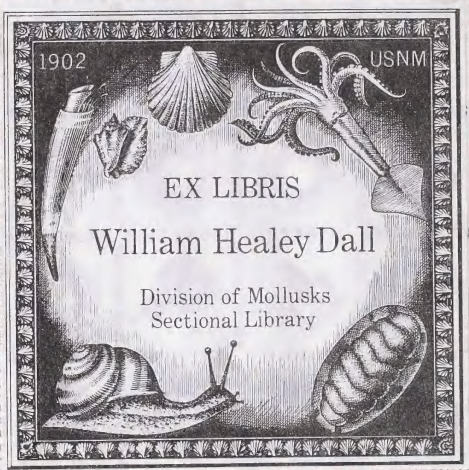

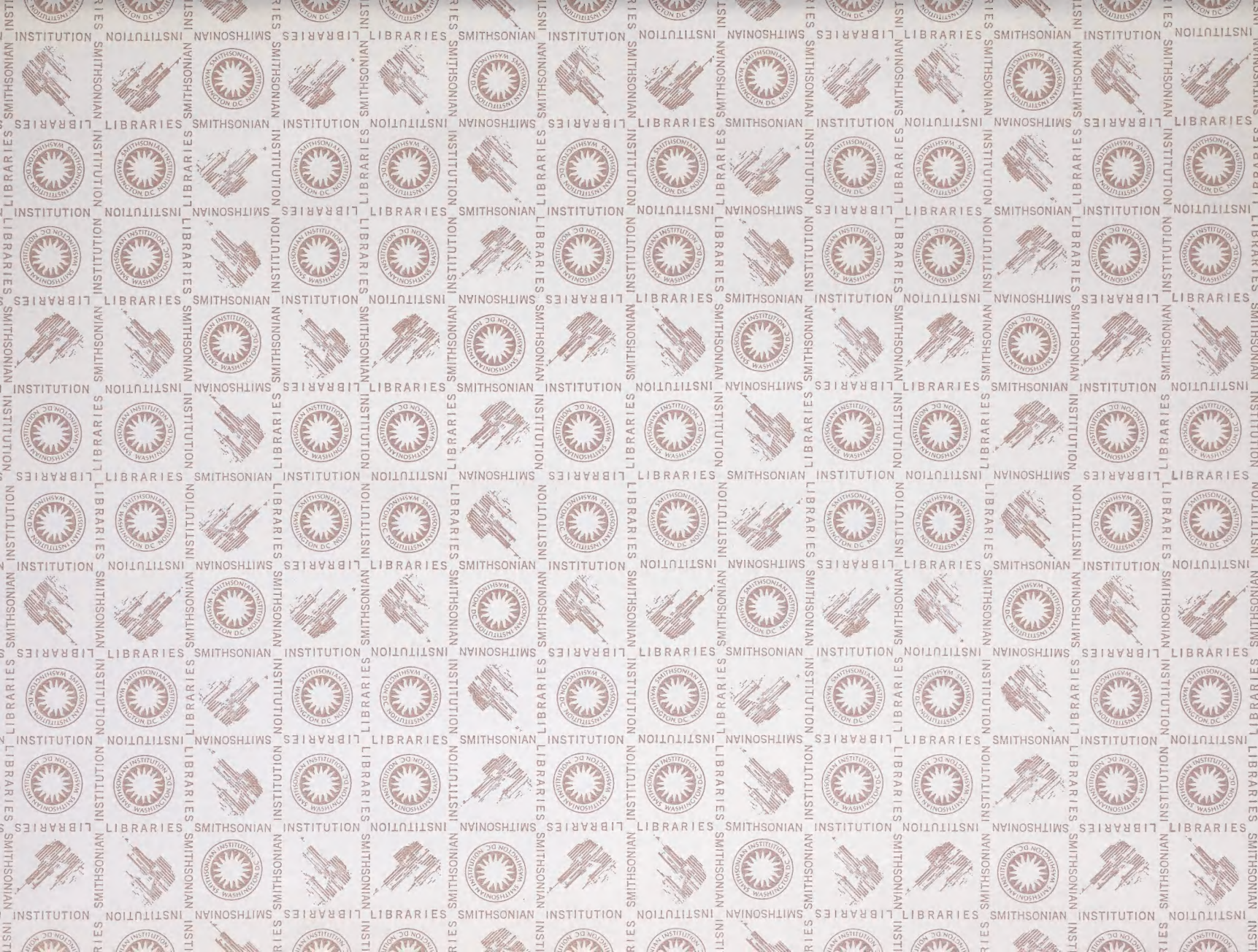
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EXPERIMENTAL STUDIES
ON
NUCLEAR AND CELL DIVISION
IN THE
EGGS OF CREPIDULA

BY
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PLATES XLIII–LIX

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EXPERIMENTAL STUDIES ON NUCLEAR AND CELL DIVISION IN THE EGGS OF CREPIDULA.

By Edwin G. Conklin.

CONTENTS.

Introduction.

Many problems of development, heredity and evolution center in the study of the causes and nature of differentiation, and particularly of the early differentiations of the egg. Some of these differentiations have been traced back to very early stages of development without finding their real beginnings, and indeed it is clear that a definite sequence of morphological differentiations must be preceded at every stage by a definite organization, *i. e.*, differentiation and integration, of the developing substance, even though this antecedent organization be only one of molecules and atoms. But many, and indeed most morphological differentiations arise in the course of development by a process of epigenesis, or creative synthesis, from other differentiations more or less unlike in kind. The great problem of development is to determine, as far as may be possible,

the sequence and origin of differentiations and the extrinsic and intrinsic factors by which they may be modified. Much has already been accomplished in tracing the sequence of morphological differentiations to earlier and earlier stages of development, but relatively little has been learned as to the nature and causes of differentiation itself. By the observation of purely normal processes one sooner or later comes to a place beyond which he can make little if any progress in the study of such problems, but the history of biology in the last twenty-five years has shown that much may be learned by the comparison of normal and abnormal processes, and especially by the combination of observations of normal processes with experiments which may be varied indefinitely.

In two previous publications (1897, 1902) I described in detail the normal development of the gasteropod, *Crepidula*, particularly as regards the phenomena of cell-lineage and of nuclear and cell division. The present work comprises the results of a series of about 260 separate experiments, extended over a period of more than ten years, on the eggs of *Crepidula plana*, the species which was principally used in my previous work. The eggs of this animal afford unusually fine material for the study of the normal processes of development, but they are less favorable for experimental work and for the following reasons: (1) Fertilization takes place within the oviduct, and eggs capable of development cannot be obtained until after they are normally fertilized and laid. (2) The laid eggs are inclosed in capsules within which they undergo the whole of their embryonic development, and when removed from these capsules they do not continue to develop normally for more than three or four days. (3) The rate of development is so slow, about one month being required to reach the stage of the free-swimming larva, that it is difficult to rear larvæ from eggs which have been subjected to experiment. But while these conditions make this material unfavorable for experimental studies on fertilization or the development of larval or adult organs, they do not seriously interfere with experimental studies on cleavage and the early differentiations of the egg. On the contrary the eggs of *Crepidula* are peculiarly favorable for such studies. In no other egg with which I am acquainted is it possible to study the details of cell organization so satisfactorily as in this one. The eggs may be obtained in large numbers and may be stained and mounted entire so as to show minute details of cell polarity, the structure and division of nuclei, centrosomes and cytoplasm, and the relation of cell growth and division to differentiation. In such a case the advantage of the study of whole eggs over that of sections is very great; one can see at a glance the relative positions of all cell constituents, and of all the cells in the cleaving egg, and one may readily study and compare these minute details in hundreds of eggs, whereas this could be done in the case of sections only by the most laborious methods and then only in a relatively small number of eggs. Of course sections have also been used in cases where it seemed necessary in order to answer questions which were left more or less uncertain by the evidence derivable from whole preparations.

In general the method of procedure was to take eggs in a given stage of development, subject them for a definite time to altered conditions, and then return them to normal conditions where they were allowed to remain for varying lengths of time. As just indicated most of the eggs were fixed and stained according to the methods described in my previous papers on *Crepidula* (1897, 1902). In practically all cases the eggs experimented on were carefully compared with others of the same laying kept under normal conditions. For descriptions and figures of the stages in the normal development of *Crepidula* reference should be made to the papers just mentioned.

The present work will deal with the following topics in the order named: (1) Abnormalities found in nature. (2) The cleavage of isolated blastomeres. (3) The effects of pressure on nuclear and cell division. (4) Effects of electric currents of varying strengths. (5) Effects of abnormal temperature. (6) Effects of ether. (7) Effects of decreased oxygen tension in the medium. (8) Effects of carbon dioxide. (9) Effects of hypotonic solutions. (10) Effects of hypertonic solutions.

I. Abnormalities Found in Nature.

(Plates XLIII, XLIV. Preparations 942–945.[1])

On the whole there are remarkably few cases of abnormal eggs in nature; probably not more than one egg in a thousand shows any abnormality whatever. In plates XLIII and XLIV are shown the principal types of such abnormalities. Most of these, as for instance figs. 2–5, 9, 11–13, 15, and probably 18–26 are due to pressure. Since the eggs are laid in very thin and delicate capsules and are crowded close together, about 175 eggs being found in each capsule, it is really surprising that a larger number of eggs do not show the effects of pressure.

The most usual effect of pressure during mitosis is the formation of a lobe of cytoplasm opposite one or both ends of the amphiaster (figs. 2, 12, 13, 20). Such a lobe, as I have maintained in a previous paper (1902), is an evidence of reduced tension in the cell membrane at the points opposite the ends of the spindle. More recently, I (1912) have observed that lobes may be formed opposite the poles of the *future* spindle, while the cell is still in a resting condition; such a condition is shown in figs. 15 and 19, and indicates that the axis of the future spindle is already marked out in the resting stage preceding division, by points of reduced tension in the cell membrane. Whether this is the chief or only cause of the orientation of the spindle in the cell, cannot at present be affirmed or denied, but it seems very probable that it is at least one of the factors determining such orientation. Associated with the formation of these lobes the spindle itself is sometimes shifted toward one pole or the other so that its equator no longer lies in the typical plane of cell division. In fig. 13 the mitotic figure has been moved so that one of the daughter nuclei lies in the division

[1] See Catalogue, p. 559.

plane while the other is far away from that plane. In fig. 9 the entire spindle has been moved into one of the daughter cells, and the division plane between the cells has cut off a cell containing two daughter nuclei from one containing none. In this case a *Zwischenkörper* or mid-body is present in the plane of division, although the entire mitotic figure lies to one side of that plane. This seems to indicate that the mid-body may be formed, or at least may persist, independently of the mitotic figure.

Other abnormalities of division which are probably due to pressure, are found in figs. 22–25; here the number of macromeres is increased from four to six, seven or eight, and this is probably due, as experiments show, to pressure in the vertical axis during the third cleavage. Such increase in the number of macromeres is not to be interpreted as the result of an "anachronism of cleavage" (Roux), *i. e.*, to the substitution of a later cleavage for an earlier one, nor are the eight blastomeres so formed comparable to those of the 8-cell stage of the normal egg. *On the contrary this cleavage is one not represented in normal eggs but is a new one which has been intercalated, and though in point of time it is the third cleavage, it is morphologically like the first and second cleavages and not like the third cleavage of the normal egg; it gives rise to no micromeres and serves merely to increase the number of macromeres to a number larger than four.* In subsequent cleavages each one of these macromeres gives rise to three micromeres (ectomeres) exactly as if it were one of the four normal macromeres. From the directions of these divisions and from the size and quality of the resulting cells it is perfectly evident that the micromere formation from each of these macromeres takes place exactly as in a normal egg. Each macromere which reaches the animal pole gives off three micromeres (ectomeres). In figs. 23 and 24 where six macromeres reach the animal pole, and one lies far below, near the vegetal pole, there are six micromeres of the first set ("quartet"); in fig. 24 five of the macromeres are dividing in a læotropic direction to form the micromeres of the second set, and it is evident from the position of the nucleus and sphere that the sixth and smallest macromere will also give rise to a micromere of the second set when it divides. In fig. 25 it is evident from the positions of the spindles in the five macromeres that the third set of micromeres are being formed, and although it is not possible in this figure to identify certainly all the micromeres of the first and second sets, it is probable that there are six micromeres of the second set, and six of the first, each of the latter having budded off in læotropic direction a small "turret cell." In fig. 26 there are but three macromeres, two of which have given rise to three sets of micromeres, whereas the third macromere (*D*) is dividing for the first time.

In several cases in which more than four macromeres are present the nuclei of some of the cells are either irregular in form or multiple in number. Thus in fig. 22 there are several scattered nuclei in four of the cells (three at the animal, and one at the vegetal pole), and in fig. 24 there is a triaster in one cell and double nuclei occur in two micromeres and in one macromere. These are probably karyomeres or partial nuclei, due to polyasters, such as are seen in fig. 16, and

such an egg would probably divide at once into seven or eight cells, the number of macromeres in this case being due immediately to abnormalities in the mitotic figure rather than to pressure. In this case the initial causes of these triasters and polyasters can only be conjectured; probably cell division was suppressed by pressure, while centrosomal and nuclear division went on, as will be explained later.

A few other types of abnormal cleavage occurring in nature have been observed. The eggs shown in figs. 6, 7, 8, have not divided into several macromeres, but the yolk has remained undivided while two separate micromeres have been cut off at the animal pole of the single macromere. The mitoses by which these cells were formed were abnormal, as is shown by the character of their nuclei, but the positions of the centrospheres, and the relation of the cells themselves to the macromere, as well as the position of the spindle in fig. 6, indicate that these small cells may correspond to the first and second micromeres of the normal egg. If this be true we have here another evidence of the fact that *the number of micromeres is proportional to the number of macromeres, in every case three and only three micromeres being cut off from each macromere, i. e., with one macromere the micromeres are formed in sets of one, with two in twos, with four in fours, with six or eight in sixes or eights, etc.* In this connection it is interesting to note that Bigelow (1902) found in the egg of *Lepas* that the single macromere of that egg gave rise successively to one first, one second and one third ectomere and then to one mesomere, and he concluded that such a type of cleavage might be derived from the "quartet" type by the suppression of the first and second cleavages of that type.

Fig. 18 shows an egg in the 4-cell stage in which the nuclei are entirely lacking in two of the cells, although other parts, such as sphere and mid-body, are present. I do not know how such an abnormality has arisen but it may be that the nuclei were extruded after the division of the cells. On the other hand fig. 21 shows an egg in which the nuclei continued to divide in normal rhythm although the cell body did not, three of the spindles of the third cleavage being found in one cell, without proper orientation, while the spindle in the other cell is normal in position.

Finally figs. 27, 28, 29 represent a few eggs and embryos from a great number in which the yolk mass or endoderm was not overgrown by the micromeres, thus giving rise to exogastrulæ. The lot of eggs showing these abnormalities was found in a place where the sea water was diluted with fresh water, and the experimental study of the effects of diluted sea water on the eggs shows that this is the probable cause of these abnormalities.

These are the only types of abnormalities which I have found among the many thousands of normal eggs which I have studied; among these, however, are representatives of several forms of abnormalities of cell division which have occurred in my experiments.

II. Cleavage of Isolated Blastomeres.

(Plate XLV. Exps. 855–857, 887–892, 921–931, 957–959.)

The cleavage in *Crepidula* belongs to that type which I once (1897) called "determinate," in which every blastomere has a definite prospective significance, giving rise to a definite part of the embryo and larva. Is this "determinism of development" due to intrinsic causes, to the character of the oöplasm in a blastomere, or to environmental influences acting on the blastomere, such, *e. g.*, as the interrelation between different blastomeres, etc.? Is the "potency" of a blastomere of the *Crepidula* egg as limited as is its "prospective significance"? This is a problem to which much attention has been devoted in the case of the development of other animals, but although most of my observations on this subject in the case of *Crepidula* were made many years ago, nothing has hitherto been published regarding it. It is undesirable at this place to review the very extensive literature on this subject, which Korschelt and Heider call *Das Determinationsproblem*, and for an excellent discussion of the work on this problem prior to 1902, reference is made to Korschelt und Heider, *Lehrbuch der vergleichenden Entwicklungsgeschichte, Allgemeiner Theil, II Capital.*

In general, the blastomeres of the egg of *Crepidula* are closely adherent to one another so that it is difficult to separate them without injury; nevertheless in the anaphase of the first and second cleavages the blastomeres become so rounded that they touch one another only by relatively small surfaces and at this stage they may sometimes be separated by pressure or by shaking, without injuring the cells. Sometimes such isolation is brought about by diluted sea water, or by ether, but the injury to the cells in such cases is usually considerable so that the subsequent development of such isolated blastomeres is complicated by such injury. In an egg with such a definite cleavage pattern as that of *Crepidula*, where the position, size and histological character of many cells are highly characteristic, the study of the cleavage of isolated blastomeres is of more than ordinary interest. It is here possible to determine with certainty whether the cleavage goes forward in strictly partial fashion, whether it undergoes certain modifications although still remaining partial in other respects, or whether the blastomeres return to the condition of the unsegmented egg after their isolation.

In practically every instance it is found that the isolated blastomeres round up and in subsequent cleavages the cells come into contact with one another forming contact surfaces which are often different from those of the normal egg; this flattening of cells against one another usually involves slight rotation of the cells and a consequent slight shifting of the cell axes with reference to the original cell complex. But with this exception the cleavage of isolated blastomeres is strictly "partial." The general direction of cleavages, and the size and histological character of the blastomeres are in all cases like those of the normal egg. If isolation takes place in the 2-cell stage each ½ blastomere divides equally,

and in a slightly læotropic direction, into two macromeres (fig. 30, *A*, *B*) and each of these macromeres then gives rise to a first micromere of normal size and quality by dexiotropic cleavage (fig. 31, 34), then to a second one by læotropic cleavage (fig. 35), and then to a third one by dexiotropic cleavage (fig. 37), exactly as in normal eggs. If isolation takes place in the 4-cell stage each ¼ blastomere (macromere) gives off a first micromere by dexiotropic, a second by læotropic, and a third by dexiotropic cleavage (figs. 32, 33, 34). If one of the four macromeres is separated from the other three, the ¾ blastomeres, if uninjured, behave as they would if the egg were still entire, except that they move together to close the gap left by the missing macromere (figs. 39, 41, 43, 44), unless one or more of them are injured, in which case the gap may not be completely closed (figs. 36, 40, 42). Every ¼ macromere whether isolated or joined with one or more others, gives rise to three micromeres of typical size and quality by divisions which are typical in direction.

In all cases the relative sizes of macromeres and micromeres are approximately the same as in normal eggs, and the micromeres are always free from yolk spherules. The direction of the spindle axes, the direction and extent of rotation of the cells in telokinesis, and the final positions of nuclei and spheres in the cells are always the same as in normal eggs, with the single exception, mentioned above, that owing to the closing of the gap left by the removal of one or more macromeres the direction of cleavage, with reference to the whole, is slightly altered. This closing of the gap is caused chiefly by the turning in or rotation of entire cells and the axis of each of these cells, as marked by its nucleus and centrosome, remains unchanged.

The subdivisions of the micromeres of typical eggs are also highly characteristic; the first division of the first micromeres is læotropic and very unequal, the small peripheral products being the turret cells ($1a^2$–$1d^2$), while the large central products are the apical cells ($1a^1$–$1d^1$). The first division of the second micromeres (2*a*–2*d*) is dexiotropic and approximately equal. The first division of the third micromeres (3*a*–3*d*) is læotropic and slightly unequal, the peripheral product being smaller than the central one. In all of these respects the subdivisions of the micromeres of partial eggs are wholly typical; in the first division of the first micromere the spindle is eccentric in position and the division gives rise to a small "turret cell" ($1a^2$–$1d^2$) whether the partial egg consists of one, two or three quadrants (figs. 33, 35, 37–44); in the first division of the second set of micromeres the spindles are not eccentric and the resulting daughter cells are approximately equal (figs. 37, 41, 42, 44); in the first division of the third set of micromeres the spindles are again eccentric in the cell and the peripheral daughter cells are smaller than the central ones (fig. 43).

Later subdivisions of the micromeres of partial eggs are also entirely typical for each quadrant. The second division of the apicals ($1a^1$–$1d^1$) is unequal giving rise to a larger peripheral cell, the "basal cell" ($1a^{1,2}$–$1d^{1,2}$) and a smaller "apical cell" ($1a^{1,1}$–$1d^{1,1}$); at the same time the right half of each micromere of the

second set gives off a small central cell, the "tip cell," and a large peripheral one; while the left half of each of these micromeres reverses this, giving off a small peripheral cell from a large central one; there is thus formed an "ectodermal cross" with its center at the animal pole and with an arm running out over each macromere and ending in the "tip cell" which is larger in quadrant *D* than in any of the others (see stippled cells, fig. 43). In all of these respects the ¾ eggs shown in figs. 43 and 44 are entirely typical though they lack all the cells of one quadrant. Finally in the partial egg shown in fig. 43 the macromere 4*D* has given rise to the mesentoblast, 4*d*, which has divided into right and left halves, M^1 and M^2, in a manner entirely typical; on the other hand when the macromere *D* is lacking no mesentoblast cell is formed. Other partial eggs caused by diluted sea water are shown in figs. 135, 144, 151, 155. These also illustrate the principle that the development of isolated blastomeres is entirely typical so far as the cleavage of each quadrant is concerned. The cleavage from the 4-cell stage on is, in the language of Roux, "a mosaic work consisting of four independently developing vertical pieces." These results are in essential agreement with those reached by Roux (1892), Chabry (1887), Crampton (1896), Wilson (1904), Conklin (1905) on the cleavage of isolated blastomeres in eggs of animals having "determinate" types of cleavage. Of course where individual blastomeres are not peculiar in size and quality it is not possible to determine to what extent the cleavage of isolated blastomeres is atypical. In *Crepidula* the individual peculiarities of the blastomeres are so marked that we have here one of the best possible opportunities for the study of this problem.

III. Effects of Pressure on Cleavage.

(Plates XLVI, XLVII, XLVIII. Exps. 849–854, 884–886, 893–920, 1000–1004.)

Another method of studying the determinism of cytoplasm and nuclei in the cleavage stages is to subject the eggs to pressure so that the spindle axes and cleavage planes are turned out of their normal positions. Such experiments were performed by Pflüger, Roux, Born, and O. Hertwig, on amphibian eggs; by Driesch, Morgan, and Ziegler, on echinoderm eggs; by Ziegler, on ctenophore eggs; and by Wilson, on the eggs of *Nereis*, etc. In this way the early cleavage furrows may be displaced from their normal positions so as to form linear series or flat plates of cells, the distribution of oöplasmic substances (yolk, cytoplasm, etc.) to the blastomeres may be greatly changed and the nuclei which in normal eggs would have gone into micromeres may be caused to go into macromeres. Driesch and Hertwig in particular have maintained that these pressure experiments demonstrate the totipotence of the cytoplasm and karyoplasm, since normal development may result from eggs in which both of these plasms are abnormally distributed. Nevertheless Driesch has shown, and most of the other investigators named above confirm this conclusion, that the structure of the cell protoplasm differs in different axes, and that the cleavage of isolated blasto-

meres from the animal pole differs from that of blastomeres from the vegetal pole. On the other hand practically all investigators agree that the nuclei of the cleavage cells are not only totipotent but that they are qualitatively all alike. The following experiments on the effects of pressure on *Crepidula* eggs confirm in the main the conclusions reached by previous investigators.

1. *Modifications of the Cleavage Pattern.*

The eggs of *Crepidula plana* are laid in thin, transparent, membranous capsules; about 175 eggs are found in each capsule, and the walls of the capsules are usually flattened together in one plane so that opposite walls are in contact at the middle of the capsule while the eggs are crowded into the ring-shaped space at its periphery. Owing to the crowding together of the eggs in these flattened capsules it sometimes happens that eggs which have not been subjected to experiment show the effects of pressure, as previously mentioned, but such modifications are relatively rare in a state of nature. In most of the experiments the eggs were subjected to pressure within the capsules; in some instances (Exps. 897, 900–908, 910, 911, 916–920) they were removed from the capsules before being subjected to pressure. Whether the eggs were pressed within the capsules or after removal from them the results were essentially the same. In general eggs which had been removed from the capsules were more frequently broken and injured than when they were pressed within the capsules. The capsules, or freed eggs, were placed under cover glasses, or between glass slides in dishes of fresh sea water, or they were placed in a Ziegler compressorium through which a current of water was kept running, and were allowed to remain in such positions for from four to eighteen hours. After removal of pressure the eggs were either fixed at once or were allowed to develop under normal conditions for periods of time varying from one to sixteen hours. Varying degrees of pressure were thus applied in different axes and for various lengths of time to eggs in different stages of development. In most cases pressure was applied until the spherical eggs became flattened disks, and it was surprising to note how much flattening the eggs would bear without bursting.

In cases where pressure was applied during the maturation of the egg, and parallel with its chief axis, the polar spindle may remain near the center of the egg (fig. 68) and the polar bodies may be abnormally large, as in fig. 45 where the second polar body is as large as an ordinary micromere; or a large "yolk lobe" may be formed at the vegetal pole. In cases where large polar bodies are formed their nuclei are usually correspondingly large. Not infrequently all division is stopped during pressure and I think that in such cases the pressure is most frequently in the direction of the normal spindle axis.

The results of pressure during the cleavage of the egg are essentially the same, so far as cell division is concerned, as during the maturation, *i. e.*, the spindles may be turned out of their normal positions, the relative sizes and positions of the daughter cells may be altered, the cleavage may be suppressed,

or lobes may be formed on the surface of the cells, usually opposite the poles of the spindle. Figs. 46, 47, 49 show eggs in which the first cleavage was made unequal; figs. 47, 48, eggs in which lobes were formed near the vegetal pole; fig. 50 is an egg which was pressed in the direction of the second spindle axis and in which cleavage was halted in one of the macromeres (*CD*) while the other continued to divide; fig. 57 is an egg, pressed in the same direction as fig. 50, in which the four macromeres are arranged in linear series; fig. 51, an egg which has divided into three cells, each with several nuclei, probably the result of a triaster. In no instance, however, have I found evidence that triasters or tetrasters may be formed as the immediate result of pressure. On the other hand they are frequently due to the continuance of nuclear and centrosomal division after the suppression of cell division, the result being that two or more nuclei and centrosomes are left in a single cell body and in subsequent mitoses triasters, tetrasters or polyasters may be formed. Figs. 69–72, 74 show eggs in which the second cleavage spindles are out of their usual positions, and in which the division of the cell body is partially or entirely suppressed. These eggs were subjected to the action of the electric current, while being pressed between graphite-plate electrodes, but it is probable that the abnormalities shown are due to pressure rather than to the electric current. In fig. 73 an egg is shown in which one of the first two blastomeres contains two nuclei, while the other one contains a well formed spindle without any chromatin. This is probably the result of pressure during the first cleavage, whereby both daughter nuclei were forced into one blastomere, leaving only a centrosome in the other blastomere, as in fig. 13. Figs. 69, 72, 75 represent successive stages in the development of three different eggs in which the first cleavage furrow was suppressed, probably by pressure between the graphite-plate electrodes.

Pressure during the third and fourth cleavages in the direction of the chief axis of the egg leads to some very interesting modifications of the cleavage type. The micromeres which normally lie on top of the macromeres and which are about 1/20 the volume of the latter, may thus be caused to lie in nearly the same plane as the macromeres and to equal them in size. Of course this is brought about by the turning of the spindles from a position nearly parallel with the egg axis to one at right angles to that axis. It has been a matter of surprise to me to see how difficult it is to bring about this change in the position of the spindles and how much the eggs must be flattened in order to accomplish it. Even under the greatest flattening which the eggs will stand without bursting, the spindles still preserve a little of their original slant, the end of the spindle nearer the polar bodies being at a little higher level than the opposite end. Consequently in most of my experiments the cells which are formed at the apical (central) ends of the spindles are at a little higher level than those at the outer ends, and the former are usually smaller than the latter, though containing yolk and being much larger than typical micromeres. Fig. 52 represents an 8-cell stage in which the third cleavage in quadrants *C* and *D* was nearly normal; in *A* and *B*

the "micromeres" $1A^1$, $1B^1$ are very large and contain yolk; in the macromere $1A^2$ the nucleus and cytoplasm lie on the animal pole side of the cell, on the other hand $1B^2$ is entirely cut off from the animal pole. A study of the later cleavages of such eggs after pressure has been removed shows that only those cells which preserve a cytoplasmic area on the animal pole side give rise to micromeres; therefore, $1B^2$ being cut off from the upper half of the egg is incapable of forming micromeres and is in the condition of the cell $1D^2$ in figs. 23 and 24. Figs. 53–55 are 8-cell stages in which some of the "micromeres" are much larger than usual and contain yolk. It is interesting to observe that all such "micromeres" are dividing synchronously with the macromeres and in general in the same direction as the macromere from which they were derived. In fig. 53, the "micromeres" $1A^1$, $1B^1$, $1D^1$ are dividing in a direction similar to that of the normal micromere (see fig. 56, 1*c*) but the spindle is so placed in each cell that the peripheral portion instead of being a small "turret" cell will be a large yolk-containing cell; the position of the spindles in the macromeres $1B^2$–$1D^2$ is læotropic, which is characteristic of the fourth cleavage (formation of second quartet) but in $1A^2$ the position of the spindle is dexiotropic. In fig. 54, the normal position of the spindles is reversed in $1A^1$, and $1A^2$. In fig. 55 the spindle positions are all dexiotropic, the reverse of normal, though the position of centrosome and nucleus in $1B$ indicates that the direction of cleavage in this cell will be læotropic.

In fig. 76 is shown an egg in which pressure in the direction of the chief axis of the egg, during the third cleavage, led to the formation of eight macromeres, each of which has now given off one micromere; the direction of division at the third cleavage was prevailingly læotropic, at the fourth cleavage dexiotropic in quadrants, *C* and *D*, and prevailingly læotropic in *A* and *B*. Fig. 77 shows another egg pressed in the direction of the chief axis after the formation of the first quartet, and during the formation of the second and third quartets; the abnormalities in the latter are indicated by the lettering and arrows between cells. Fig. 78 represents an egg which was pressed between graphite-plate electrodes for two minutes, during which time a current of 5 mil. amp. was passed between the electrodes, the egg was then left in sea water under normal conditions for 22 hours; there are six macromeres and twelve micromeres; four of the macromeres and three of the micromeres are dividing, the stage corresponding to that of the formation of the second set of micromeres in normal eggs. The pole at which the micromeres form differs in different blastomeres and is widely different from that of normal eggs. Fig. 79 shows an egg which was pressed for ten minutes between graphite-plate electrodes between which a current of 5 mil. amp. was passed, the egg was then left in normal sea water for 16 hours. The second set of micromeres are being formed and the first set are dividing, but the egg shows plainly the effects of pressure in the direction of the chief axis.

The egg shown in fig. 56 was evidently pressed after the formation of the first set of micromeres (1*a*–1*d*) which are quite normal; on the other hand, the

macromeres of the second set are larger than normal, and $2a$ and $2c$ contain a quantity of yolk. In figs. 58 and 59 pressure in the direction of the chief axis of the egg has led to the formation of the first set of micromeres between the macromeres and in the same plane with them. In fig. 59 each of the eight cells has divided or is dividing in a dexiotropic direction (with the possible exception of the cells in quadrant D). The direction of this cleavage is therefore like that by which the first set of micromeres are formed, and there are thus formed almost simultaneously eight protoplasmic cells at the animal pole which may be regarded as micromeres of the first set. In fig. 58 the 16-cell stage is completed; it consists of four "micromeres" of the first set, each of which by læotropic division has given off peripherally a micromere, two of which ($1b^2$ and $1d^2$) are much larger than normal, while the other two ($1a^2$ and $1c^2$) have been given off on the vegetal side; the macromeres have also given rise to "micromeres" of the second set, which contain yolk and are much larger than usual; this division was læotropic in A, B, C, but dexiotropic in D. Fig. 60 represents an egg which was pressed 4 hrs. and freed 6 hrs. Quadrants A, B, D are normal except that the "turret" cells in quadrants B and D ($1b^2$, $1d^2$) are larger than normal; in quadrant C the turret cell ($1c^2$) is also larger than normal and the micromere of the second set ($2C^1$) is as large as a macromere. In all cases the direction of division is normal. Fig. 61 is normal except in quadrant A; evidently the first set of micromeres was formed in typical fashion but the second division of macromere A was nearly equal, giving rise to $2A^1$ and $2A^2$, the former of which has again divided into $2A^1$ and $2a^1$, but the latter ($2A^2$) gives rise to no micromere of the second set, all of its second micromere material having gone into its sister cell $2A^1$. Fig. 62 represents an egg like the preceding one in which the first division of macromere C was nearly equal; the upper one of the macromeres thus formed ($1C$) has divided, giving off an apical cell $1c^1$; the elongation of this cell and the spireme in its nucleus indicate that it will soon cut off a turret cell at its peripheral pole; the position of cytoplasm, nucleus and centrosome in $1C$ indicates that this cell is about to give off a micromere of the second set ($2c^1$), whereas the same indications show that the macromere $2C$ has given off a micromere of the second set ($2c$), but none of the first set; in other respects this egg is normal. In fig. 63 there are six macromeres, owing to the fact that the first division of A and D was nearly equal; each of these six macromeres has produced a micromere of the first set, while one (C) has produced a micromere of the second set. The positions of the nuclei and spheres in $1B$, $1A$, $1D$, $1D^1$ indicate that the next division in these cells will be læotropic, and will give rise to micromeres of the second set; on the other hand in $1A^1$ it will be dexiotropic. In fig. 64 the conditions are much like those shown in fig. 58, viz., the first micromeres have given off, or are just forming the "turret" cells; two of these, $1b^2$, $1c^2$, lie at the lower pole; the second micromeres are larger than usual and contain yolk; one of these, $2a$, was formed by a dexiotropic division, the others by læotropic division as in normal eggs. In fig. 65 quadrants C and D are approximately normal, $2B$ has been crowded under the

ectomeres, while A gave rise to a micromere of the first set, much larger than usual, which has divided equally into $1a^1$ and $1a^2$, the latter being much larger than in the other quadrants; the second division of A was a nearly equal one, the second quartet cell $2A^1$ being a macromere. In fig. 66 the axis of pressure was at right angles to the egg axis, B having been crowded under A, and D under C; the first, second and third quartets and their subdivisions are normal, except that some of the cells are slightly displaced. Fig. 67 represents an egg which was flattened in the direction of the egg axis; the three sets of micromeres and their subdivisions are approximately normal; the usual ectodermal cross is present (its cells are stippled); the fourth quartet cell (4*d*) has been formed and is larger than usual. My material showing the effects of pressure does not include stages older than the egg shown in fig. 67, owing probably to the retardation of cleavage in eggs which have been much pressed; in these same experiments many eggs which were pressed less, and are therefore normal, have reached the 64–72-cell stage. In some of the experiments the eggs were under pressure for 18 hrs. and were fixed 4 hrs. after being freed; in others they were pressed 16 hrs. and freed 16 hrs., yet in no instance has the cleavage of abnormal eggs advanced beyond the 42-cell stage.

2. *Differential and Non-Differential Cleavages.*

Owing to the slow rate at which pressed eggs of *Crepidula* develop, it is not possible in this case to determine the morphogenetic potency of the various atypical blastomeres which are thus produced. However the cleavage patterns of these pressed eggs show that a meridional cleavage, approximately parallel with the chief axis, divides the egg substance in such manner that every blastomere so formed subsequently divides as a normal macromere. If four macromeres are present, as is the case in normal eggs, each gives rise to three ectomeres; if pressure is applied during the third cleavage, so that five, six, seven or eight such macromeres are formed each still gives rise to three ectomeres; if the pressure is applied after the first set of micromeres are formed so that the macromeres divide equally in the fourth cleavage and by a vertical plane, each of these macromeres in subsequent division gives rise only to micromeres of the second and third sets. If "micromeres" are formed at the third cleavage, which are much larger than usual, as in figs. 53, 59, 62, 63, *et al.*, these cells do not become ectomeres in their entirety but they cut off protoplasmic ectomeres at the animal pole from yolk-containing entomeres at the lower pole. If the third cleavage is rendered equatorial, each of the cells of the animal pole gives rise to three sets of ectomeres, but those at the vegetal pole produce no ectomeres. If the cleavage plane is oblique, between meridional and equatorial, the cells so formed may give rise to a varying number of ectomeres, and in general only macromeres which reach the animal pole produce three ectomeres, while the farther the macromeres are removed from that pole the smaller the number of ectomeres which they produce.

These facts are important with reference to one of the greatest problems of

embryology, viz., the factors of embryonic differentiation. The earliest differentiations of the fertilized egg are those which occur during cleavage; the differentiations of cleavage lead to the formation of cells which are qualitatively and quantitatively different, and if the causes of unequal and dissimilar cleavages can be found, an important step will have been taken toward the solution of the causes of embryonic differentiation. The facts presented above suggest that the substances of the egg are stratified from the animal to the vegetal pole, and that only oöplasm of a definite sort is given off in the ectomeres (micromeres) leaving material of a different sort in the entomeres (macromeres).

On the other hand when the more fluid substances which normally lie at the animal pole are thrown by centrifugal force into one of the first two blastomeres leaving only yolk and a small amount of cytoplasm in the other blastomere, the cell with the greater quantity of fluid substance gives rise to only three ectomeres, while that with the smaller quantity also gives rise to three ectomeres (see Conklin, 1912). Evidently therefore it is not merely the quantity of fluid substance in the macromere, nor the stratification of the more dense and less dense oöplasmic substances, which determines whether or not three ectomeres shall be formed.

It seems necessary to conclude as Lillie (1906) and Morgan (1910) have done that the heavier or lighter substances of the egg, which may be stratified by centrifugal force, are not morphogenetic substances, but are mere inclusions in the real morphogenetic material. So far as yolk is concerned it is known that it is a relatively dense inclusion which may be stratified at the distal pole of the centrifuged egg, or may even be thrown out of the egg without seriously modifying the morphogenetic processes. The same is true of the lighter oily or watery substances which may be stratified or thrown out at the proximal pole of the centrifuged egg. When both these denser and lighter inclusions are eliminated there is left the clear substance of the middle zone in which the nucleus and centrosome usually lie; and even the most of the material of this middle zone may be transferred from one of the first two blastomeres to the other, without interfering with the development of either blastomere. In what part of the cell, then, does the polarity, symmetry, and morphogenetic organization reside?

In *Crepidula* a nucleus, centrosome and sphere, plasma membrane and probably a minimal quantity of the protoplasm of the middle zone (hyaloplasm) must be present in a blastomere if it is to develop at all, and it must be in some one or more of these parts, or in their relations to one another, that the organization resides. In my experiments the one visible thing which is left unchanged in these centrifuged eggs is the axial relation between nucleus, centrosphere and cortical layer. It is extremely difficult to move the mitotic figure by centifugal force,—apparently it is anchored to the cortical layer by threads of denser protoplasm,—and it is possible that a framework of such threads connects nucleus, centrosphere and cortical layer, and thus preserves the axial relation mentioned above. Such threads of denser protoplasm may possibly

correspond to the "ground substance" of Lillie (1906), but it is to be noted that I do not regard these threads as the seat of polarity and organization, as I understand Lillie to do, but merely as a framework which preserves the typical axial relations of nucleus, centrosphere and cortical layer. But it is insufficient to say that the organization of the egg determines the position of the mitotic figures and consequently the cleavage pattern. More specifically the central spindle, or netrum, usually forms at right angles to the cell axis (the axis passing through the centrosome, nucleus and mid-body) and in the plane which separates the nuclear halves or gonomeres. Owing to the movements of telokinesis in the cleavage of *Crepidula* the cell axes undergo regular changes with respect to the axis of the egg as a whole; the direction of telokinetic movements is such that the centrosomes always move toward the animal pole and toward the point where the previous cell constriction began. Consequently successive cleavage planes are not at right angles to one another, but the cleavages take place in a "spiral" manner. A further consideration of this subject is reserved for another paper.

Equality or inequality of division is due in part to the localization of different oöplasmic substances, and in part to local reductions in the tension of the cell membrane. Indeed telokinetic movements, and consequently the direction of division as well as the equality or inequality of daughter cells, are associated with local variations of tension in the cell membrane. O. Hertwig (1892) long ago maintained that the spindle lies in the direction of least resistance in the cell; certainly this is not the principal factor which determines the position of the cleavage plane, as Jennings (1896), Zur Strassen (1896) and I (1897) have shown. Doubtless the spindle lies in the axis of least resistance in many cases, but the position of the spindle does not make that axis the one of least resistance. It is probably owing to local reductions in the tension of the cell membrane that there is an axis of least resistance, and these reductions of surface tension are not caused by the position of the spindle, but *vice versa*. Indeed the characteristic movements of the cell contents in all stages of division (prokinesis, metakinesis, telokinesis) and consequently the initial direction of the subsequent spindle, as well as the equality or inequality of cleavage, are probably associated with local variations in the tension of the cell membrane. But more important than any of these factors are the mitotic movements of the cell contents, which begin with the dissolution of the nuclear membrane in the prophase and which carry the spindle into certain definite positions in the cell and there bring about constriction of the cell body in a particular plane which usually passes through the equator of the spindle.

The position of the spindle, then, is due to at least four factors viz: (1) The separation of daughter centrosomes at right angles to the cell axis and in the plane which separates the gonomeres. (2) Telokinetic movements by which the cell axis undergoes regular changes with respect to the axis of the egg as a whole. (3) Local reductions of tension of the cell membrane in certain axes, or increase of tension in other axes, by which an axis of least resistance is established

in the cell. (4) Mitotic movements of the cell contents, which cause the spindle and the surrounding plasma to move into certain axes and positions in the cell.

The quality of a cell, that is, the character of the cell substance which it contains, appears to be due in the main to the relation between the spindle axis and the stratification or localization of cell substance at the time of its formation, *i. e.*, to the way in which the cleavages cut through the different cell substances. *The morphogenetic value of a blastomere appears to depend primarily upon the kind of protoplasm, apart from mere inclusions, such as oil and yolk, contained in the cell, though it is known that the nuclear substance may influence the prospective potency of a blastomere in cases of regulation or restitution.*

In the ascidians, as I have pointed out elsewhere (1905) "the prospective significance of a blastomere is a function of its position" (Driesch) only because of its relation to the organization of the egg as a whole—because of the particular morphogenetic substances which go into it—and not because of its position relative to other blastomeres. The experiments described above, on the effects of pressure and of centrifugal force, on the cleavage of the eggs of *Crepidula*, indicate that the same principles are involved in this case also, though the cytoplasm of this egg contains a larger amount of inclusions or non-morphogenetic materials and is more capable of regulation than is the case with the ascidian egg.

So far as the nucleus is concerned these pressure experiments indicate that the divisions are non-qualitative. When the third cleavage spindle is forced into an equatorial plane and the cells divide meridionally instead of latitudinally, as in normal eggs, the subsequent development of the extra macromeres thus formed may be quite normal, each giving rise to three ectomeres, just as if the normal number of macromeres were present. Therefore the organization of the nucleus is not such that each of its divisions has a specific morphogenetic value; on the contrary the nuclear divisions are non-differential and in this respect, non-morphogenetic. Of course the nucleus has a definite structure, polarity, symmetry, etc., but its division by mitosis is such that the daughter nuclei are alike.

IV. Effects of Electric Current.

(Plates XLVIII, XLIX. Exps. 995–999, 1100–1123, 1140–1142.)

Many authors beginning with Fol (1873) have emphasized the resemblance between the mitotic figure and the lines of force in a magnetic field. Nevertheless attempts to identify the forces at work in the two cases have largely failed. Errera (1890) was unable to find any effect of electricity or magnetism upon cell division in *Tradescantia*. Nevertheless Ziegler (1895) and Gallardo (1896) maintained that the mitotic figure was the expression of a bipolar force, and the latter held that this force was probably electrical in nature. Hartog (1902, 1905, 1907) also held that the mitotic figure is an expression of a bipolar force —his mitokinetic force—which he likens to, but does not identify with, electricity. Hartog, as well as Gallardo in his earlier work, held that the poles of the spindle were heteropolar, bearing opposite signs, though Gallardo has since

abandoned this view and now (1909) thinks that both poles of the spindle are alike and are positively charged, while the chromosomes are negatively charged.

Schücking (1903) and Delage (1908) found that artificial parthenogenesis may be induced by an electric current, but they made no study of the effects of the current on cell structures. R. Lillie (1903) showed that isolated nuclei and spermatozoa, suspended in cane sugar solution, through which an electrical current is passed migrate with the negative stream, while cells with voluminous cytoplasm migrate with the positive stream. He concluded therefore that the colloidal particles composing the chromatin carry negative charges, the cytoplasmic particles positive charges.

In four subsequent papers on the physiology of cell division R. Lillie (1905, 1910, 1911[1], 1911[2]) points out many parallels between electric and mitotic phenomena. In the first of these papers (1905) he holds that "the disposition and relative positions of many colloid aggregates in the cell, especially the chromosomes and chromatic filaments during mitosis, indicate that mutual electrostatic attractions and repulsions play an important part in determining their position and movements." The formation of the spireme and of the equatorial plate, as well as the arrangement of the chromosomes with respect to one another and to the poles of the spindle in amphiasters and triasters was simulated by the use of floating magnetized needles exposed to the attractive or repellant action of magnetic poles. Such models were held to indicate that the astral centers have a repellant action on the chromosomes and therefore, since chromosomes are negatively charged aggregates, these astral areas must also be negatively charged. In the last of the papers cited Lillie (1911[2]) concludes "that by taking account of the changes of potential resulting from alterations in the permeability of electrically polarized membranes certain characteristic phenomena of mitosis are susceptible of consistent physico-chemical explanation."

Pentimalli (1909, 1912) and McClendon (1910) confirm Lillie's conclusion that the chromatin bears a negative charge, but McClendon is unable to confirm Pentimalli's observation that in the root-tip of the hyacynth the chromosomes are carried through the cell walls toward the anode. He finds however that the basophile substances of the cell, as well as the mitotic figures as a whole, migrate toward the anode. Roux (1891) found that a "morphological polarization" of the egg substances of different animals, not unlike the stratification produced by centrifugal force, was caused when an electric current was passed through the egg. All of these effects are undoubtedly due to convection currents within cells. Baltzer (1908, 1911) has criticized the views of Hartog and Gallardo, showing that triasters and tetrasters in echinid eggs demonstrate that the poles cannot have opposite signs and that spindles may form where there is no chromatin. In his later work Gallardo (1909) admits that all poles are alike in sign, but denies that a true spindle may be formed where there is no chromatin. He affirms that spindles without chromatin are only apparent, and never lead to cell division. One may admit the truth of the latter half of this statement without granting that the first half is true. Certainly cells do not usually divide if chromatin is

lacking, though this has been observed by Ziegler, Wilson and others, but the initial spindle (netrum) is formed without the presence of chromatin in the spindle, and perfect spindles of full size may occur without any trace of chromatin, as Boveri (1897) and Baltzer (1908) have shown, and as is figured in figs. 73 and 90 of this paper.

The fundamental difficulty with Gallardo's theory as a theory is its inconsistency. If the poles of the spindle are of like sign, as he now holds, and the chromatin is of opposite sign, the movements of chromosomes toward the poles in the anaphase find an explanation, but not their movements into the equatorial plate. To explain the latter, Lillie supposes that astral centers and chromatin are of the same sign, viz., that both bear negative charges, but this would not explain the movements of the chromosomes toward the asters in the anaphase, for two daughter chromosomes not only separate from each other, but they cling to other chromosomes and as plates move close to the poles; such movements cannot be explained by assuming that centrosomes and chromosomes bear like charges and hence repel one another. Leduc (1902, 1904) holds that the mitotic phenomena cannot be explained as the result of electric phenomena, whereas they may be the result of diffusion phenomena. About the same time (1902) I maintained that astral radiations are diffusion streams, and the results of later work have not caused me to change this opinion. Mitotic phenomena are complex and they are doubtless due to several factors, rather than to a single one. Damianovich (1907) believes that several forces may be at work, viz. electricity, polarity, diffusion phenomena, surface tension, hydrostatic polarity and probably different chemical energies; and Gallardo (1909), to whose review I am indebted for a knowledge of this paper, apparently approves this conclusion. Stomps (1910) holds that the separation of chromosomes is due to the formation and growth of vacuoles between the daughter chromosomes. But it is evident that the phenomena of mitosis and their causes must be more specifically analyzed before we can construct theories as to the mechanics of the process.

If the mitotic figure is the expression of electrostatic charges carried by colloidal particles composing the chromosomes, centrosomes and cytoplasm it seems probable that the figure would undergo modifications of form or orientation if an electric current were passed through it, and such modifications should throw light upon the nature of the charges carried by different portions of the figure. With this thought in mind I have subjected the eggs of *Crepidula*, during cleavage, to the action of a constant current. In my first experiments with the electric current, which are catalogued at the end of this paper as Nos. 995–999, the eggs, still within their capsules, were placed in a paraffine trough about 5 cm. long, 1 cm. wide and ½ cm. deep, containing a small quantity of sea water. In Exp. No. 1120 the trough was 12 cm. long and about 2 cm. wide and 1 cm. deep. In the paraffine wall at each end of the trough a boot-shaped porcelain electrode was imbedded, with the toe projecting into the trough. Dilute copper sulphate

was put into the legs of the boots and into this the wires from one or more dry batteries dipped. The impressed voltage varied from ¼ volt to 1 volt, but the amperage was not measured. Under the conditions described it is uncertain to what extent the current actually went through the eggs. When the trough was filled with eggs and the amount of water was very small many of the eggs were penetrated, but in general the resistance of the eggs was so much greater than that of the surrounding water that many of the eggs were left in a normal condition, and it may be assumed that few of the eggs were penetrated by the full strength of the current.

Nevertheless many eggs show by certain unmistakable signs that they were penetrated by the current. Not infrequently eggs were killed outright and even fragmented or disintegrated; in some cases they were coagulated together into a single mass. Where the injuries were less gross, but where the eggs were undoubtedly killed there is no distinct separation between protoplasm and yolk, and the chromatin of the resting nuclei, the chromosomes and spindle fibers of division stages have dissolved more or less completely (figs. 80–86). In some cases the chromosomes have disappeared from the amphiaster, leaving the spindle fibers and polar rays intact (fig. 90). Where the injury was less severe the substances of the egg may be stratified by the current, the nuclei and cytoplasm being driven to one pole while the yolk remains at the other. Strands and streamers of cytoplasm may extend through the yolk or over its surface, thus indicating the direction of movement, as in figs. 88, 91. This dislocation of oöplasmic substances is in many respects similar to that produced by centrifugal force. In this respect my results confirm those of Roux, Pentimalli, McClendon, *et al.* This stratification is the result of convection currents within the cell and it throws little if any light upon the nature of the mitotic figure. For this reason it seemed desirable to employ weaker currents and at the same time to take such precautions as were possible to insure the current penetrating the eggs. In Exp. 1120 a current of 1 mil. amp. was passed through the trough between porcelain-boot electrodes for 45 min., but this current was so weak that it produced no effect whatever upon the eggs.

Later experiments with the electric current were performed under the following conditions: The eggs, either within the capsules or after they had been liberated, were placed on a graphite plate, which had previously soaked in water, the water was drained off and a second graphite plate, similar to the first, was laid over the first, the two being separated by cover glasses of a thickness about equal to the diameter of the eggs. Each plate was connected with the poles of a dry battery, or of several dry batteries in circuit. The strength of the current used varied from 50 mil. amperes to 1 mil. ampere, but the exact area covered by the eggs on the graphite plate was not measured in every case. In general, however, the eggs covered an area about 3 cm. square. With a current of from 40–50 mil. amperes which was used in experiments 1100–1103, the eggs were coagulated almost at once and stuck fast to the anode. In all later experiments, the current

was reduced to at most 7 mil. amperes, and in several cases to 5, 2, or 1 mil. ampere. The duration of the current varied from 15 minutes to 1 minute and the eggs so treated were fixed either at once or from 1 to 60 hours after they had been returned to dishes of sea water. All of these experiments are catalogued as Nos. 1100–1143, at the end of this paper.

Many eggs placed between the graphite plates show the effects of pressure; many of those shown on Plate XLVIII are of this sort. The abnormalities in the positions of the spindles shown in figs. 68–74 are duplicated in the pressure experiments, while the dislocation of cells shown in figs. 75, 78, and 79 are also very similar to those shown in the pressure experiments. Figs. 76 and 77 represent eggs which were pressed between glass plates, whereas all the other eggs shown on Plate XLVIII were subjected to the electric current between graphite plates.

Many of the eggs used in these experiments were killed and their membranes dissolved; others, on the other hand, developed normally. The earlier stages of cleavage usually suffered much more than the later ones. In general, the most characteristic effect of the current is found in the massing of chromatin within the nuclear vesicle, as in synapsis, or in the failure of the daughter nuclei to become vesicular. Indeed the latter may remain perfectly dense spheres. In some cases, the polarity of the egg or of the cleavage cells seems to be lost and the usual sharp separation between yolk and cytoplasm disappears. Sometimes the direction of the spindle is abnormal. The eggs shown in figs. 80–93, Plate XLIX, illustrate some of the most common abnormalities observed in eggs subjected to the electric current. In all of these eggs, the boundaries between cytoplasm and yolk are but faintly marked; the nuclear membranes are usually very thin or altogether lacking and the chromatin is massed within the nucleus or scattered in clumps through the cytoplasm where it dissolves and disappears, figs. 80–86. In some cases tetrasters are present (fig. 87), but these are probably due to other causes than the electric current. In a few instances, chromosomes have entirely disappeared probably by solution, from the amphiaster leaving the achromatic parts of the latter quite perfect (fig. 90); in other cases, the achromatic part of the amphiaster as well as the chromosomes may be undergoing solution (figs. 86, 91, 92). Figs. 91 and 92 show eggs in which the position and the direction of the mitotic figures are abnormal. Finally, one frequently finds eggs like fig. 93, in which the micromeres no longer form a flat plate of cells covering over the macromeres, but rounded and irregularly scattered cells. Such conditions, which are probably the same as those named *framboisia* by Roux, are not limited to eggs treated with the electric current but may be found in almost all cases of abnormality appearing in the later cleavage and they probably represent the rounding up and separation of micromeres which were formed normally.

The abnormalities shown in Plates XLVIII and XLIX represent the principal types of abnormalities found among eggs which have been subjected to the electric

current. Among the many thousands of eggs so treated very few show any trace of modification of the structure or orientation of the amphiaster. Where the current is strong enough to produce any modification in the structure or orientation of the mitotic figure it is usually strong enough to dislocate yolk, cytoplasm and resting nuclei, as well as entire amphiasters, and to kill the eggs. In such gross changes entire spindles, centrosomes as well as chromosomes, are carried toward one pole, which judging by the work of Lillie, Pentimalli and McClendon is the anode. *In these experiments there is no indication whatever that the poles of the spindle bear electric charges differing in sign from the chromosomes nor that the spindle fibres or astral radiations represent lines or chains of force between differently charged poles.* If the forces which are at work in the amphiaster are electric, this could scarcely be the case, and it seems probable therefore that the so-called "mitokinetic" force is not electric.

On the other hand I find evidence that the spindle fibres are composed of relatively tough material and that they may be bent, distorted or displaced by centrifugal force from their normal positions without losing their identity. In this regard my results differ from those of F. R. Lillie (1909) and agree with those of Morgan (1910). Furthermore I find that the chromatic sap of the nucleus forms the interfilar substance of the spindle and escapes, when the nuclear membrane dissolves opposite the centrosomes, into the areas around the centrosomes, and that it radiates from these areas and from the entire spindle into the cell body by a process which resembles protoplasmic flowing in amœboid cells. By treatment with various chemical reagents, as well as with electricity or increased temperature this flowing may be stopped and the processes or radiations withdrawn into the central mass of archiplasm (hyaloplasm and chromatic sap). The astral radiations are therefore expressions of diffusion streams, rather than of electric lines of force. Of course it is probable that there is a difference of potential between the electric charges carried by the particles of this chromatic sap, and the colloidal particles of the cell body, but it also seems probable that the spindle figure and its radiations are due in the first instance to the escape and diffusion of nuclear substances into the cell body, rather than to the electric potentials of these substances.

Whenever two or more astral systems are present the chromosomes at first take up positions between them. Between two such centers the chromosomes form a rectilinear equatorial plate, and when many centers are present the area around each is bounded by a line of chromosomes, the whole forming a complicated pattern of hexagonal or polygonal areas, each inclosing a centrosome and bounded by chromosomes. Such a condition would be produced if centrosomes and chromosomes carried like charges, as Lillie maintains. This condition is followed quickly by a stage in which the daughter chromosomes separate from one another, and, as daughter plates, approach the centrosomes. This movement might, conceivably, be due to the daughter chromosomes carrying like charges and consequently repelling each other; however there is, on this hypothesis, no

apparent reason why chromosomes are drawn together at the poles of a bipolar figure, on the other hand they should repel one another and thus be scattered through the cell in positions of equilibrium between the poles and the other chromosomes.

In conclusion, I do not find that the phenomena of spindle formation nor of chromosomal movement can be consistently explained by Lillie's or Gallardo's hypotheses. I find no evidence in favor of these hypotheses in experiments in which an electric current is passed through dividing cells. On the other hand I find abundant evidence in all my experiments that the phenomena of karyokinesis and also of cytokinesis are associated with diffusion phenomena between nucleus, centrosome and cell body.

V. Effects of Abnormal Temperature.

(Plate L. Exps. 960–961, 1170–1177.)

Many experiments have been made on the influence of temperature on development; among more recent investigations those of Driesch (1893), O. Hertwig (1890), Delage (1901), Greeley (1902), Schücking (1903), R. Lillie (1908) on echinoderms, and of Rauber (1883), O. Hertwig (1896), O. Schultze (1894, 1899), Lillie and Knowlton (1897), Bataillon (1903) on amphibians, must be mentioned. Of work which deals primarily with the influence of altered temperature on cell structure and division, the most important is that of O. and R. Hertwig (1887), who found in the sea urchin egg that a large increase of temperature led to polyspermy, suppression of astral rays, even those in connection with the sperm nucleus, lack of growth on the part of the sperm nucleus, eccentricity of first cleavage spindle and unequal cleavage (*Knospenfurchung*). Driesch (1893) found that increased temperature led to a reduction of the surface tension of the blastomeres of sea urchin eggs, to more or less separation between the blastomeres and to suppression of micromere formation. Sometimes the blastomeres of the 8–16 cell stages separated into two groups, thus forming twins. If the temperature were increased during gastrulation, exogastrulae resulted. O. Hertwig (1896) found that increased, as well as decreased, temperature led to the suppression of cleavage and development at the vegetal pole of the frog's egg.

The influence of decreased temperature on eggs has been studied by O. Hertwig (1890) who found in sea urchin eggs that a temperature of 2° to 3° C. for a half hour led to the formation of a large reception cone; if the eggs were then warmed, a high degree of polyspermy resulted. Cold caused the disappearance of astral rays, which quickly reappeared on warming; it also caused the degeneration and complete disappearance of the first cleavage spindle and of the polar rays, while the chromatic part of the amphiaster remained unaltered; when such eggs are warmed, the spindles and polar rays appear again and division is completed normally. By long action of cold the chromosomes are affected and form a reticular or vesicular nucleus. Morgan found that segmentation might be started in unfertilized eggs of *Arbacia* by freezing the water in which they

were placed. Sala (1893) and Zur Strassen (1898) found that the eggs of *Ascaris megalocephala* might be caused to fuse together by cooling them to 3° to 4° C.

My own work on the influence of altered temperature on the cleavage processes of *Crepidula* are limited to ten experiments,—nine on the effects of increased and one on the effects of decreased temperature. At the time the experiments were made the normal summer temperature of the sea water at Wood's Hole was about 22° C.; however the temperature of the water in the finger bowls in which the eggs were developing normally, varied from 22° to 27°.

In the experiments with increased temperature the eggs were taken from finger bowls at room temperature, in which the thermometer registered from 22° to 27°, and were put into sea water varying in the different experiments from 33° to 38° C. They were kept in the warm water from ¼ hr. to 4 hrs.; some of the eggs were then fixed at once, while others were put back into the finger bowls in water which varied with the room temperature from about 22° to 27°, where they were left for periods of time varying from 3 hrs. to 15 hrs. The records of each of the experiments are given in the catalogue at the end of this paper.

The eggs thus treated showed many notable changes in structure,—indeed in no other experiments recorded in this paper were the modifications so great and so interesting. That these modifications are not due merely to heat coagulation is shown by the partial or complete recovery of the eggs when returned to normal conditions. However owing to the relatively small number of experiments, I have not been able to analyze these modifications as thoroughly as could be wished. This I hope to do in a later paper, as I am now engaged in extending these experiments.

In the experiments with altered temperature, as in all the others, the greatest changes are produced when the eggs are in some phase of kinesis at the beginning of the experiment, while stages of interkinesis are affected relatively little. The early stages, also, are more affected than the later ones. The most general results of increased temperature are: (1) Reduction of surface tension and consequent irregularities in the outlines of the eggs; owing to local reductions of surface tension the type of polar-body formation or of cleavage may be greatly changed (figs. 94, 95). (2) Segregation of archiplasm (hyaloplasm and chromatic nuclear sap) into distinct areas, separate from the clearer plasma (enchylema); withdrawal of the archiplasm of the astral radiations into the central areas of the aster and into the division walls between cells (figs. 94–99). (3) Profound changes in the orientation and structure of the mitotic figures; loss of all spindle fibers and centrosomes; scattering of chromosomes, which are often thrown entirely outside the mitotic figures and even outside the cytoplasmic areas (figs. 97–99). (4) Formation of numerous karyomeres from these scattered chromosomes; indeed by slight increase of temperature almost every chromosome may be caused to remain distinct from every other one, and to give rise to a separate chromosomal vesicle.

Only one experiment was made on the effects of decreased temperature on

the cleavage processes in the eggs of *Crepidula*. In this experiment, dishes of sea water containing eggs in early cleavage stages (1–8 cells) were placed on ice in a refrigerator for from 4 to 40 hours, and were then fixed at once after removal from the refrigerator. The temperature of the water in the dishes was a little above 2° C. Figures 100–102 represent eggs which were on ice 16 hours; figures 103–105, eggs which were on ice 40 hours. Cleavage in these eggs was greatly delayed if not entirely stopped; after 40 hours on ice many of the eggs were still in the 4-cell stage. Spindle fibers and astral rays were present, though perhaps less distinct than in normal eggs.

The most characteristic feature of eggs which have been on ice from 4 to 16 hours is the great distinctness of the spheres which, in resting stages, appear to be bounded by a definite membrane (fig. 101), and which stain so deeply that they look very much like nuclei. In division stages, the spheres are surrounded by coarse, chromatic granules, the sphere granules (S. G., fig. 100 *et seq.*), which are possibly homologous with mitochondria. In the eggs which were 40 hours on ice, the spheres have lost their distinctness and in their places are masses of coarse sphere granules (figs. 103–105). Apparently the centrosomes in the resting stages have also disappeared and further experiments are necessary to determine whether they also have broken up into granules.

VI. Effects of Ether.

(Exps. 800–803, 818–819, 877–878, 1180, 1181.)

In order to test the effects of anæsthetics on the kinetic phenomena of cell division, varying quantities of ether were added to sea water in which eggs were placed. With a small quantity of ether acting for a short time no effect was noticeable. Thus ½ per cent. to 1 per cent. of ether acting for ½ hr. to 1 hr. produced no visible changes. However ½ per cent. acting for 24 hours caused the nuclei to become more densely chromatic and the spindle fibers and astral rays of the mitotic figure to disappear. 1 per cent. acting 5½ hrs. or even for 16 hrs. caused very few if any changes; the distribution of cytoplasm and yolk, and the character of nuclei and centrosomes, whether in rest or division, are apparently normal. However 1 per cent. acting for 25 hrs. produces many changes; in some cases the cleavage is very irregular and the chromatin is clumped in the resting nuclei. In division stages, many chromosomes are scattered and clumped. Nevertheless spindles and spindle fibers are present and are apparently normal in structure and function. On the other hand 3 per cent. ether acting for ¼ hr. produced profound changes in the distribution of yolk and cytoplasm. The cytoplasm is intermingled with the yolk and *vice versa*, and cytoplasm is carried in along the whole cleavage plane between macromeres. The chromosomes in mitosis are often scattered along the spindle, the spindle fibers have disappeared, and telokinetic movements are stopped. When 3 per cent. ether was allowed to act for 3 hrs. and the eggs were then placed in normal sea water

for 6 hrs., the results are the same as in the preceding experiment, except that large sphere granules are present and are scattered through the cell and surround the nuclei. Chromatin is clumped within the resting nuclei; chromosomes are also clumped in the spindles. Centrosomes, spindle fibers and astral rays may be present, but mitosis is very irregular, polyasters often being present; in other eggs spindle fibers and astral rays disappear. These results are, in general, similar to those obtained by Gerassimoff (1892), on *Spirogyra*, and by Häcker (1900) and Schiller (1909) on the segmenting eggs of *Cyclops;* in all of these cases the spindle fibers were caused to disappear by the use of ether, but whereas these authors observed that the division continued by a kind of amitosis after the disappearance of the spindle, I have seen no evidence of real amitotic division in *Crepidula*. Irregularities in the movements of the chromosomes with the consequent scattering of chromosomes along the entire length of the mitotic figure does sometimes lead to a chromatic connection between daughter nuclei. Such modified mitoses, which are suggestive of amitosis, are found in many of these experiments and are described more fully on pages 535, 548, 550 and 557.

VII. Effects of Decreased Oxygen Tension.

(Plate LI.)

In view of the fact that the centrosomes and the surrounding spheres move regularly during telokinesis to a free surface of the cell where the sphere substance spreads out under the cell membrane, it seemed desirable to determine whether this movement is due to oxidations at the surface of the cell and consequently to the presence of free oxygen in the surrounding water. Accordingly, the oxygen tension of the water was reduced in two ways, first by boiling the water in flasks or test tubes to drive off contained gases, after which the tubes were stoppered and cooled before the eggs were placed in them, second by running a current of purified hydrogen gas through a series of wash bottles in which eggs were placed.

1. *Boiled Sea Water.*

(Figs. 106–109, 112–114, 116. Exps. 935–940, 947–953, 1010–1022.)

Pure sea water was boiled vigorously for a few minutes in order to drive off contained gases, but not so much as to lead to any appreciable concentration of the salts. Flasks or test tubes of this water were carefully closed with rubber stoppers which dipped into the water and consequently left no air space in the tubes. The water was then cooled to the temperature of the water in the aquaria and the eggs were gently introduced into the tubes. In thirteen experiments of this kind (Nos. 935–940, 947–953) the tubes were left unstoppered and eggs in stages varying from 1 cell to 24 cells were put into the tubes and left in them for periods varying from 4 to 48 hours. Eggs which had been 48 hours in the boiled water were generally becoming abnormal and some of them were dead, but all eggs which were in the tubes from 12 to 24 hours were normal in form, or nearly

so, though development had been much retarded. In twelve other experiments (1010–1022), in which the tubes were carefully stoppered after the eggs were introduced, development was not only retarded but, in many cases, completely stopped. In these eggs, nuclei and nucleoli are larger than usual, which may be explained by the duration of the resting stage (interkinesis). The chromatin within the resting nucleus is usually clumped into a nucleolus-like mass and the spheres have lost their definite outlines and consist of large, scattered granules (figs. 106–109, 112–114). Mitosis may be halted at any stage for an indefinite period, the spindles and chromosomes remaining distinct, while the centrosomes become larger than normal and the polar rays grow indistinct and finally disappear entirely (figs. 107, 109). When mitosis has been halted for a long time, as in the egg shown in fig. 116 which was in a stoppered tube of boiled sea water for 48 hours, the spindles shrink in size and stain deeply, while the chromosomes form a distinct ring around the spindle.

2. *Atmosphere of Hydrogen.*

(Figs. 110, 111, 115, 117, 118. Exps. 879–883, 1023–1029.)

Hydrogen was generated in the well known Kipp apparatus and a stream of the gas, after being purified and washed, was allowed to bubble through the sea water in a flask containing the eggs to be studied. In this way, most of the air in the water and flask is replaced in a short time by hydrogen.

The first effect of such treatment is a slowing of division; after 4½ hours, there were 1-cell and 2-cell stages present in the flask, whereas in the control all the eggs had passed beyond these stages; after 7½ hours, there were many 1-cell to 4-cell stages present in the flask, whereas in the control all had reached the 24-cell stage. Development in an atmosphere of hydrogen is normal as far as it goes; the direction, size and quality of cell division and the direction of movement in telokinesis are not affected, but it is quite evident that after a short time the eggs cease altogether to develop in an atmosphere of hydrogen. In another experiment (No. 1023), hydrogen was allowed to bubble for 2 hours through a wash bottle containing eggs and the bottle was then left open to the air for 2 hours. Most of these eggs appeared quite normal, though a few showed swollen nuclei; however, development had been stopped. In other experiments (Nos. 1024–1026), hydrogen was run through bottles containing eggs for 1 or 2 hours and the bottles were then stoppered and the eggs left in them for 3, 5, 18, or 20 hours. When the eggs were in an early stage at the time of the experiment, development was stopped; in one case (No. 1026), where the eggs were in an advanced stage of cleavage when the experiment began, development progressed normally until normal gastrulæ were formed 20 hours after. This one experiment indicates, what many others prove, that the early stages of cleavage are much more sensitive to environmental changes than are the later ones. In all cases in which development is stopped for some time, nuclei and nucleoli become

much larger than normal, and the spheres and centrosomes consist of large, scattered granules; if the development has been halted during mitosis, the spindles, centrosomes, and chromosomes remain distinct but the polar rays disappear, being drawn into the central portion of the amphiaster (figs. 110, 111, 115, 117). If development has been stopped for many hours (18–48 hours), cytoplasm may move toward the vegetal pole along the first and second cleavage planes (fig. 116), while in other cases yolk may appear around the centrosomes nearest the animal pole (fig. 118).

Finally, by the aid of Dr. A. P. Mathews, I was able to employ in certain experiments (Nos. 1027–1029), atmospheres composed of known quantities of air and hydrogen. In one case (No. 1027), the proportions were 60 vols. of hydrogen to 40 vols. of air; in two others (No. 1028, 1029), the proportions were 70 vols. of hydrogen to 30 vols. of air. In all of these experiments development was stopped within a few cleavages, though the eggs remained in the bottles from 12 to 24 hours.

To sum up, it may be said that the first effect of reduced oxygen tension is the slowing of development and of cell division, and as a result of this retardation, the resting nuclei, which continue to imbibe materials from the cytoplasm, become very large and achromatic; at the same time, the nucleoli grow larger than normal, as is always the case when the resting period is prolonged. The nuclei and nucleoli thus come to resemble the germinal vesicle and germinal spot of immature egg cells, and I believe that this resemblance is a real, and not merely an apparent one. The spheres of resting stages also lose their definite outlines and are resolved into large granules which are more or less scattered. In division stages, mitotic figures may persist for many hours (18–48 hrs.); the spindle fibers remain distinct and the spindle may shrink and at the same time stain more deeply than normally; the polar radiations disappear and the centrosomes frequently enlarge. Later stages of cleavage are not as much affected as are earlier stages.

In all cases where the experiment has not been prolonged more than 24 hours, when eggs are returned to normal sea water development is resumed and the nuclei and cells become normal in appearance. In some cases the micromeres cease to form a one-layered epithelium, and become individually rounded; collectively, they form a mass more than one cell thick, as I reported in a former paper (1902, p. 102). Later work has shown that this result is exceptional rather than usual, and that it occurs in many other experiments in which eggs are degenerating (*framboisia* of Roux).

I conclude therefore that the presence of oxygen in the medium is not the chief cause of the movements of telokinesis.

VIII. Effects of Carbonic Acid.

(Plate LII. Exps. 1163–1169, 1169a, 1169b.)

Carbonic acid was first employed by Delage (1902) to produce artificial parthenogenesis in starfish eggs, and it has since been employed by many other investigators for this purpose. A cytological study of its effects on eggs has been made by Godlewski (1908). Loeb (1906) and Godlewski (1908) found that membrane formation in unfertilized echinoderm eggs could be called forth by this substance. Godlewski observed that in CO_2 solutions parthenogenetic mitoses are not preceded by nuclear growth, and that in strong CO_2 solutions fertilized eggs rarely segment, though nuclei may continue to divide, thus giving rise to cells with many nuclei. Later these nuclei may fuse together producing synkaryonts (Strasburger, 1907), which may subsequently divide by bipolar or multipolar mitoses; the size of the plasma field which subsequently cuts off around such nuclei is proportional to the size of the nuclei.

Only a few experiments were tried to learn the effects of carbonic acid on the cleavage processes in *Crepidula* but they yielded some very interesting results, and they call for an extension of these experiments.

Normal sea water was charged with CO_2 in the well-known "sparklet siphon" bottle, and equal parts of this charged sea water and normal sea water were poured over the eggs in finger bowls, which were loosely covered. The eggs were left in this water for from 7 to 42 hours, and were then fixed, stained, and mounted.

A large number of the eggs so treated are abnormal and some of these abnormalities are highly characteristic. Among these may be mentioned the following: All cell membranes appear unusually heavy and chromatic so that the outlines of the cells are extraordinarily distinct, at the same time these membranes are often wrinkled or separated from the cell substance (figs. 119, 130 *memb.*) and on many of the cells there are lobes, bridges, and threads (figs. 127, 130, *L*). comparable to those observed by Andrews (1898) in the so-called "spinning phenomena." The cleavage of the yolk may be entirely or partially suppressed, a phenomenon which is of frequent occurrence in many other experiments, while the cleavage of the protoplasmic part of the egg goes on in more or less normal manner. Thus in figs. 119–121, the cleavage of the yolk is entirely suppressed but several "micromeres," some of them with several nuclei and spheres, have been formed at the protoplasmic pole. In fig. 122, which has an abnormally large second polar body, the first cleavage spindle has divided the nuclei in a normal manner, but the cleavage furrow has cut down through the protoplasm into the yolk and there stopped in a "cleavage head" (Ziegler, 1903), such as is found in the cleavage of the eggs of many cœlenterates. In no other preparations of *Crepidula* eggs have I found such a type of cleavage as this and it would be interesting to learn whether this cœlenterate type could be produced in other eggs by the use of CO_2. The area marked *S* in the yolk of fig. 122 may be a sphere or "cytaster."

Cases of the suppression of yolk cleavage, with interesting results, are shown in figs. 123, 125, 126, 127, 128, 129. In figs. 123, 128, and 129, the macromere *AB* did not divide at the second cleavage, though its nucleus did. In fig. 123, the first micromere (1*ab*) formed from this undivided macromere is abnormal, all the other micromeres being normal. In fig. 125, the half of the second cleavage furrow between *C* and *D* was suppressed, while the half between *A* and *B* was abnormal in position giving rise to a large macromere *A* and a small macromere *B*, lying above *C* and *D*. Each of these macromeres has given rise to a micromere which is normal in size and appearance, though 1*c* is abnormal in position. In fig. 128, the second cleavage furrow was suppressed in both of the first two blastomeres, and at the third cleavage the two mitotic figures in each blastomere came so near each other at one pole that triasters were formed, with the result that each double macromere gave off a single micromere of the first set (1*ab*, 1*cd*), each with two or more nuclei and spheres. At the fourth cleavage, the two spindles in each macromere were distinct, one of them being directed dexiotropically, the other læotropically, and as the result of this cleavage each double macromere gave off two separate micromeres (2*a*, 2*b*, 2*c*, 2*d*), the two lying on opposite sides of the first micromere. In fig. 129, all the micromeres of the 24-cell stage are present and all are approximately normal. The fact that two nuclei may exist within the same cell and may divide in normal fashion giving rise to typical micromeres is thus plainly demonstrated. Where abnormal micromeres arise from such binucleate macromeres, it is usually due to the fact that the two mitotic figures lie so near each other that triasters or tetrasters are formed. One interesting fact brought out in this, and in other experiments, is that when the nucleus of a cell divides and the cell body does not, the two nuclei usually take up positions at opposite sides of the cell, and in subsequent divisions of these nuclei the direction of division is frequently dexiotropic in one and læotropic in the other, whereas in normal cleavage the position of the resting nuclei and the direction of division is dexiotropic or læotropic in both. In fig. 123, the nuclei 2*A* and 2*B* lie at opposite sides of the macromere, though the cleavages by which 2*a* and 2*b* were formed were not exactly at right angles to each other. In fig. 128, the direction of division in forming 2*a* and 2*c* is læotropic, in forming 2*b* and 2*d* it is dexiotropic. In fig. 129, the direction of division in forming 3*a* was læotropic, in forming 3*b*, dexiotropic. Fig. 131 represents an egg in which the direction of division in one of the first two blastomeres was at right angles to that in the other with the result that a T-shaped cleavage mass was formed. Each of the four macromeres has given rise in typical manner to three sets of micromeres and the first and second sets have subdivided, as they do in normal eggs, but the relation of these micromeres to one another is quite atypical, the micromeres being arranged in sets of three over the three contiguous macromeres, while the micromeres from the displaced macromere *D* are applied to one side of this three-cornered micromere plate.

The other figures shown on Plate LII are more abnormal than the ones just

described and are more difficult of interpretation. In fig. 124, three macromeres were formed in a row, the one farthest to the right being approximately normal; it contains one nucleus and sphere and has given off two micromeres, the first of which was formed læotropically and is now dividing, and the second is just coming off dexiotropically. The middle macromere contained a polyaster and gave off a large micromere with three nuclei, adjoining the polar bodies, and it now shows a polyaster in its second cleavage (fourth general cleavage). The macromere to the left contains a perfect achromatic spindle but no trace of chromatin; evidently a centrosome without a nucleus went into this macromere at the second cleavage, while the middle macromere received two or more nuclei and centrosomes.

A somewhat similar case is found in fig. 126; there are here three macromeres, one of which (right) contains an amphiaster and a triaster and has just given off two micromeres of the second set on opposite sides of the large micromere of the first set which was formed at the previous cleavage; another macromere (left) has just given off in dexiotropic direction a micromere of the second set, while the micromere of the first set which was formed from this macromere at the previous cleavage is now dividing and contains two centrosome and two equatorial plates; the third macromere (above) contains several centrosomes and spheres but no trace of nuclear material.

Figs. 127 and 130 show two very irregular cleavage forms, in which the cell membranes bulge out irregularly in lobes or threads; even the polar bodies in fig. 127 are connected with the contiguous cells by such threads. The very irregular cleavage shown in these two figures is probably due to these abnormal surface tension phenomena, which lead to the formation of cells which are atypical in size and position.

The figures shown on Plate LII are but a few of the almost infinite variety of abnormalities which resulted from the experiments with carbonic acid. It seems to me that *these results indicate the importance of surface tension phenomena in mitosis and development, the importance of cell walls in preventing the interference of one mitotic system with another, and the connection between localized reductions in surface tension and the direction and position of mitotic spindles.*

IX. Effects of Diluted Sea Water.

(Plates LIII, LIV. Exps. 858, 859, 870–876, 954–956, 993, 1182–1186.)

In testing the action of various changes in the environment on nuclear and cell division in *Crepidula*, a number of different experiments were made on the effects of diluted sea water. In the case of animals living in shallow water along shore, as *Crepidula* and its messmate *Pagurus* do, it must frequently happen that the density of the sea water is much reduced by heavy rains or by streams of fresh water. Do these eggs show an adaptation to such conditions? I found that a dilution of ¼ or ½ part fresh water to 1 part sea water produced little if any

effect on the eggs of *Crepidula*. However, when equal parts of fresh water and sea water were used, many modifications were produced. Almost all of the experiments enumerated above were made with a dilution of equal parts of fresh water from the tap and of the normal sea water supplied to the aquaria at Wood's Hole. In three of the experiments, viz., Nos. 859, 875, 876, 100 c.c. of fresh water were added to 50 c.c. of normal sea water. The eggs were left in the diluted sea water for lengths of time varying from ½ hour to 7½ hours and were either fixed at once or put back into normal sea water for periods varying from ½ hour to 52 hours.

Driesch (1893) found that sea urchin eggs did not segment in 30 parts normal sea water and 20 parts distilled water, though the nuclei continued to divide. Loeb (1895[1]) was able by means of diluted sea water to cause the egg membrane of the *Arbacia* egg to burst and a portion of the egg to flow out, and from such eggs double embryos developed. Schücking (1903) caused artificial parthenogenesis in *Asterias* by the use of distilled water.

The first effect of the diluted sea water on the eggs of *Crepidula* is to cause a slight swelling of the cells and to render all cell membranes very indistinct. Indeed the outlines of eggs become hazy and the macromeres sometimes separate from one another more or less completely. In all cases where eggs in dilute sea water are crowded together, they lose their individual outlines and apparently fuse together. If the diluted sea water is allowed to act for a long time or if the dilution is great (2 : 1), the cell membranes may burst. Even in extreme cases, however, the swelling of the eggs in diluted sea water is not great; when fixed and mounted the diameter of the normal egg after fertilization and before cleavage is about 136–140μ. The swollen egg rarely measures more than 144μ. After the eggs have been returned to normal sea water they again shrink to normal size, and cell membranes become normal in appearance.

The eggs shown in figs. 132–135, 137–140, 144, were placed for ½ hr. in sea water diluted with two volumes of fresh water, and were then left in normal sea water for 7 hrs. All other eggs figured on Plates LIII and LIV were treated with sea water to which was added equal parts of fresh water.

In all of these eggs, development has been delayed more or less. Eggs which were put into diluted sea water during maturation stages are frequently polyspermic (figs. 132–134); the supernumerary sperm nuclei are evidently undergoing degeneration, since they are homogeneous and stain faintly.

In considering the effects of diluted sea water on cell division, the effects on the division of the cell body will be considered first, and then the effects on mitosis. In figs. 132–135 and 145–148 division of the yolk has been completely suppressed; a very large second polar body is shown in fig. 134, and various stages in the cleavage of the protoplasmic portion of the egg are shown in figs. 145–148. The arrangement of the micromeres in the figures last named is more or less irregular, and it is not possible to tell whether three micromeres were separated from the single large macromere in sets of one, or whether the micromeres were separated from the macromere in sets of two or more. Since polyasters were present in

many cleavages of the macromere, it is not probable that the cleavages are entirely comparable to those of normal eggs.

If the first cleavage had already occurred when the eggs were put into diluted sea water, the yolk may fail to divide in the second cleavage, so that only two macromeres are present throughout the later development (figs. 137–139, 149, 150, 157), or one of the macromeres may divide and develop normally, while the other remains undivided (fig. 140). In these cases each macromere may give off micromeres in more or less normal fashion; thus one micromere of the first set is formed from each of the macromeres in figs. 137–139, and these micromeres are apparently normal in structure and position, though the macromeres from which they came contain polyasters or multiple nuclei. However in the more advanced stages of such eggs, shown in figs. 149, 150, the micromeres of the different sets cannot be identified with certainty.

In most of the eggs in which cleavage furrows have been suppressed, polyasters and multiple nuclei are present, and in many instances these are undoubtedly due to the interference of two mitotic systems, originally independent. Sometimes, however, the two mitotic systems within a single cell remain independent and give rise to micromeres which are in the main normal. Thus in figs. 157–158 the macromeres did not divide at the second cleavage, though the nuclei did. These nuclei and their mitotic figures have remained distinct and have given rise to a cap of ectomeres, which are nearly normal, though frequently the divisions by which they were formed have been bilateral rather than spiral; thus in fig. 157 the two spindles in the one macromere, which are labeled 4*C* and 4*D*, are bilaterally arranged. Both figs. 157 and 158 show that the fourth quartet cells 4*c* and 4*d* are formed simultaneously from the macromere *CD*, whereas, in the normal egg, 4*d* is formed at the 24-cell stage and 4*c* at the 52-cell stage. *The fact that the two nuclei are in the same cell has led to the synchronizing of two divisions, which are separated by a considerable space of time when these nuclei are in separate blastomeres.* Furthermore the size and structure of these cells, 4*c* and 4*d*, are alike and it is probable that both are mesentoblasts, whereas in normal eggs 4*d* is relatively small and rich in protoplasm and is the one and only mesentoblast while 4*c* is large and rich in yolk and is an entoblast. *In the absence of a division wall between C and D the typical differentiations which arise between these cells and their progeny cannot develop.*

But while the yolk cleavage is frequently suppressed in eggs treated with diluted sea water, it is sometimes, apparently, increased, since eggs may be found with more than four macromeres (figs. 143, 156). However it is probable that this abnormality is due to pressure rather than to diluted sea water. In fig. 142 the macromeres are separated and the micromeres are crowded in between them, just as in fig. 55 of the pressure experiments. In fig. 156 it appears that the egg was subjected to pressure in the direction of the chief axis after the third cleavage and before the fourth; the first set of micromeres and their derivatives are quite normal, the second set consists of large cells filled with yolk; this egg is more or less like figs. 56, 60, 64 of the pressure experiments.

Not infrequently the blastomeres of the 2-cell or 4-cell stage are separated from one another by the action of diluted sea water, and the further development of such isolated blastomeres is similar to that which occurs when the blastomeres are isolated by shaking. Thus figs. 135, 144, 151, 155 represent various stages in the development of blastomeres isolated in the 2-cell or 4-cell stage by treatment with diluted sea water; they closely resemble figs. 33, 35 and 44, where the blastomeres were isolated by shaking. In fig. 144 the macromeres *A* and *B* were separated from *C* and *D* in the 4-cell stage; each macromere has given off a micromere of the first set, which has divided normally, and the micromeres of the second set are forming in typical fashion, except that the chromosomes are scattered along the whole length of the spindles. Fig. 151 is the half of an egg in which the second cleavage furrow was suppressed, and the method of its isolation is indicated by figs. 149, 150; in both of the latter the yolk cleavage was suppressed after the first cleavage and the two macromeres were nearly separated from each other, remaining connected only by a narrow bond; micromeres have formed from these half-isolated macromeres in more or less normal manner, though I am unable to identify them individually.

In a few instances, as shown in fig. 136, the polarity of the egg may be changed, or even reversed. In this figure a polyaster was present at the first cleavage, which was also abnormal in other respects, but the most significant abnormality is found in the position of the cytoplasm, nuclei and spheres at the side of the cells opposite the polar bodies; the polarity of these cells is reversed as compared with that of normal eggs. I am unable to explain how this condition has arisen.

Finally we may consider the effects of diluted sea water on the mitotic figures. As already noted a very common abnormality is the presence of polyasters in division stages and, as a result of this, of multiple nuclei and spheres in the resting stages. Such polyasters are, in most cases, due to the suppression of the cleavage furrow and to the subsequent interference of amphiasters, originally independent.

Another abnormality of mitosis which is so common in these experiments as to be very characteristic of them, is the scattering of chromosomes along the length of the spindle, and general irregularity in the separation of chromosomes toward the poles of the spindle. Even in amphiasters which are otherwise normal the chromosomes show this irregular distribution (figs. 140, 144, 153) and of course it appears also in polyasters (figs. 135, 137, 138, 139, 147, 152). Frequently a part of the chromosomes are never drawn to the poles of the spindle, but are scattered along the connecting fibers, and when the chromosomes which have reached the poles unite to form daughter nuclei, the scattered chromosomes form a chromatic strand connecting the nuclei (figs. 141, 143, 152, 154). In many instances this chromatic connection between the daughter nuclei is strongly suggestive of amitosis (figs. 141, 143, 152, 154), although there is no doubt that it is produced in the manner stated. Similar chromatic connections between daughter nuclei, due to the scattering of chromosomes along the length of the

spindle, are found in many other experiments,—indeed this is one of the most common modifications of mitosis. This phenomenon and its significance will be considered further in the final section of this paper, pp. 550–553, 557–559.

X. Effects of Hypertonic Sea Water.

(Plates LV–LIX.)

NaCl added to sea water: Figs. 159–172, 182–192, 202–207, 209–213, 217, 220–223. Exps. 804–816, 821–832, 839–840, 843–844, 861–865, 965–990, 994, 1187.

$MgCl_2$ added to sea water: Figs. 197–200, 208, 214–216, 218, 219. Exps. 833–835, 841, 842, 845, 846, 866–868, 991, 992.

KCl added to sea water: Figs. 173, 193, 194. Exps. 836–838, 847, 847*a*.

Sugar added to sea water: Exp. 869.

Herbst's Ca-free sea water: Fig. 201. Exp. 848.[1]

The effects of hypertonic sea water upon cell division have been studied by a large number of investigators, particularly since Loeb's (1900) epoch-making discovery that artificial parthenogenesis could be induced in sea-urchin eggs by this means. Among the more important works in this field must be mentioned those of O. and R. Hertwig (1887), J. Loeb (1892, 1895[2], *et seq.*), Morgan (1894, 1896, 1899, 1900), Norman (1896), Driesch (1892, 1895), Herbst (1895, 1900), Wilson (1901, 1901[1]), Lillie (1901, 1905), Bataillon (1901, 1904), Scott (1906), Treadwell (1902), Lefevre (1907), Kostanecki (1904, 1906, 1908), Konopacki (1911), *et al.* All of the authors named found that hypertonic solutions led to a retardation or cessation of cell division, while almost all observers have agreed that nuclear and centrosomal divisions may go on after division of the plasma has ceased. Morgan finds that the most characteristic effect of hypertonic sea water is the production of astrospheres, which may appear in large numbers and subsequently fuse together into a few very large ones. He concludes that there is a definite substance (cyanoplasm) which forms the astrospheres, and that if cell division is delayed by hypertonic solutions this cyanoplasm may accumulate to form one or two suns or astrospheres.

Loeb (1906) supposes that hypertonic solutions act first in a physical way on the plasma by osmotically removing water from the egg and thus preventing cell division, without necessarily affecting the chemical processes by which nuclein is synthesized from constituents of the plasma. In such eggs chromatin may grow, chromosomes may form and divide, and astrospheres may develop, while streaming and contractility of the plasma is interrupted and cell division is stopped. With higher concentration of salts the chemical processes may also be brought to a standstill.

Further references to the literature of this subject will be made in connection with the various effects produced on the eggs of *Crepidula* by hypertonic solutions,

[1] This is classified under hypertonic solutions because its effect on cell division was similar to that of a hypertonic solution.

but I must not fail to call attention at this place to the many general resemblances between my work and that of Konopacki (1911).

In the course of this work 79 separate experiments were made on the effects of hypertonic sea water on cell division in the eggs of *Crepidula*. In 60 of these experiments the sea water was rendered hypertonic by the addition of NaCl in varying quantities; in the remaining experiments $MgCl_2$, KCl, or cane sugar were added to sea water. In all cases percentage solutions were used. After having been subjected to the action of these solutions for varying lengths of time the eggs were either fixed at once or were placed in normal sea water for a longer or shorter period before being fixed. All the eggs used in each experiment were stained and mounted, and most of them were studied, though not one in a thousand are represented in the drawings.

The results obtained in any given experiment may be extremely varied, depending to a considerable extent upon the general stage of development of the eggs, and upon their precise stage in the cycle of cell division at the time the eggs were put into the salt solution. Early stages of development are always more susceptible to any kind of environmental change than are later ones; and stages during kinesis are much more susceptible than are those during interkinesis. In particular the first and second cleavages are most easily modified and seem to be especially unstable.

My observations confirm the conclusions of Loeb and others that there is nothing specific in the action of any one of the salts named; where all other conditions are similar, essentially the same results are obtained, whatever salt is used. Therefore the results of these experiments will be described under the types of abnormalities produced rather than under the particular salts employed. With different salts, different strengths of solution are necessary to produce similar results. In ½ per cent. NaCl and 1 per cent. $MgCl_2$ all division goes on slowly, but typically (Exps. 861, 866); while ½ per cent. KCl (Exp. 847) produces certain abnormalities. In 1 per cent. NaCl (Exps. 804, 808, 988, 989, 994), ¾ per cent. KCl (Exp. 836), and 2 per cent. $MgCl_2$ (Exps. 833, 841) yolk division is stopped, especially in the first and second cleavages, while centrosomal, nuclear and cytoplasmic division may proceed slowly and more or less atypically. In 2 per cent. NaCl (Exps. 805, 809, 969, 970), 1 per cent. KCl (Exp. 837), 4 per cent. $MgCl_2$ (Exps. 842, 846) all division, nuclear as well as cytoplasmic, ceases while the eggs are in the solutions, and if left in these solutions for approximately 9 hrs. this stoppage of all divisional phenomena is permanent, even when the eggs are returned to normal sea water, although the eggs may remain alive and the nuclei may continue to grow in size.

Morgan (1899) discovered that weak solutions acting for a long time are similar in effect to strong solutions acting for a short time. My work confirms this conclusion, but only in cases where the solutions are strong enough to inhibit all divisional phenomena during the time the eggs are in the salt solutions. *If solutions are weak enough to allow any of the parts of a cell to divide, the time of*

action becomes a very important factor. For this reason a weak solution acting for a long time leads to the greatest possible departures from typical development (Exps. 808, 821, 966–968, 833, 836, etc.; figs. 195, 196, 200, 208, etc.). On the other hand eggs which have been subjected to strong solutions for a short time may afterwards develop quite normally; thus in Exps. 815 and 816, 978–980, 3 per cent. and 4 per cent. NaCl acting for 1 hr. completely stop all development for the time being, but when the eggs are afterwards put into normal sea water development goes on in a typical manner; on the other hand if these solutions act for 4 hrs. or longer all cleavage is permanently stopped even though the eggs be put back into normal sea water. This fact is shown still more strikingly in Exps. 981–987 in which 5 per cent. or 6 per cent. NaCl is allowed to act for ½ to ¾ hr. after which the eggs may develop quite typically when put back into normal sea water. From these experiments it is quite evident that the injurious effects of hypertonic solutions are due primarily to their influence on kinetic rather than on static phenomena, and particularly upon the divisional phenomena of the cell. In all my experiments eggs are much more susceptible to injury during division than during rest, and when abnormalities of division are once started they rarely, if ever, right themselves. It is owing to these facts that relatively weak solutions acting for a long time (during which developmental processes proceed in abnormal directions), lead to the most extreme forms of abnormalities.

My observations on the effects of hypertonic solutions on cell division confirm the conclusions of practically all investigators who have dealt with the subject, while at the same time they contribute certain new details and interpretations. The immediate effects of hypertonic solutions on cell division fall into one or another of the following classes: (1) Suppression of yolk division without suppression of protoplasmic, nuclear, or centrosomal division. (2) Suppression of division in yolk and protoplasm, without its suppression in nucleus or centrosome. (3) Suppression of all forms of division, without stopping nuclear growth or killing the cells. This suppression of division in each of these cases may be temporary, the division starting again when the eggs are returned to normal sea water, or it may be permanent, depending upon the strength of solution used and the time of its action. In addition to these effects of hypertonic solutions on cell division the following general effects on the different parts of the cell may be noted: (4) Shrinkage of plasma, nuclei, chromatin, and mitotic figures. (5) Formation of cytasters and polyasters. (6) Irregularities in movements of chromosomes. (7) Separation of chromatin and achromatin.

1. *Suppression of Yolk Division without Suppression of Protoplasmic, Nuclear, or Centrosomal Division.*

(Figs. 159–170, 197–208, 211–216.)

In weak salt solutions (½ per cent. KCl, 1 per cent. NaCl, 2 per cent. $MgCl_2$) division of centrosomes and nuclei may proceed slowly, while division of yolk is

suppressed and cytokinetic movements and division of the protoplasm is stopped or greatly retarded. Figs. 159–170 represent eggs which were placed in 1 per cent. NaCl in sea water for 4 hrs. and were then fixed at once. In all of the eggs shown on plate LV nuclear and centrosomal division is going on, though yolk cleavage has been suppressed.

Other cases, in which cleavage has been suppressed in the yolk while still going on in the protoplasm, nuclei, and centrosomes, are shown in figs. 195–208 and 209–223. In all of these cases the eggs were subjected to a weaker solution for a long time or to a stronger solution for a short time, after which they were returned to normal sea water. In all these eggs, which are in division stages, multipolar spindles are present, which are probably the result of the interference of originally separate spindles; while multiple nuclei and spheres are present in resting stages. Cleavage is limited entirely to the protoplasmic portion of the egg, which is thus transformed from a holoblastic to a meroblastic type, and it is interesting to observe that in many cases the micromeres formed show more or less resemblance to normal micromeres. Thus in fig. 211 the spindles are in position for the formation of the first set of micromeres; in fig. 213 a first set of micromeres, normal except in number of cells and nuclei, is present, and in fig. 212 a first and second set are present, which are also normal, with the exceptions just specified. Similar tendencies to normal micromere formation, after the suppression of the first or second cleavages of the yolk, are shown in figs. 214–218, *et seq.* In all of these cases the phenomena of nuclear division are very abnormal and *the fact that micromeres may be formed in more or less normal manner in such eggs indicates that the form of cell division may be to a certain extent independent of the type of nuclear division.*

Boveri (1897) found that when the first cleavage furrow in the egg of *Echinus* is suppressed by pressure it never reappears; two cleavage nuclei are left in the undivided egg and at the second cleavage two spindles are formed, usually vertical to the axis of the first cleavage spindle and parallel to each other; the second cleavage furrow then appears as if it were the first and two cells are formed each with two nuclei, and this process is continued in later cleavages. Wilson (1901[2]), on the other hand, found that in such eggs of *Toxopneustes* the first cleavage furrow was restored after the third cleavage and sometimes even earlier. *All my observations on Crepidula indicate that when once a cleavage furrow has been suppressed and other furrows are subsequently formed, the suppressed furrow is never restored.*

2. *Suppression of Division in both Yolk and Protoplasm without its Suppression in Nucleus and Centrosome.*

(Figs. 174, 175, 183–190.)

In salt solutions of medium strength (1 per cent. KCl, 2 per cent. NaCl, 3 per cent. to 4 per cent. $MgCl_2$) cleavage of both yolk and protoplasm is

stopped in early stages, and if eggs remain in such solutions for a considerable time (9 hrs. or more) all cleavage is permanently stopped, though the nuclei may sometimes resume division when the eggs are put back into normal sea water. On the other hand if these solutions are allowed to act on eggs for a shorter time the cleavage of the protoplasm may be resumed when the eggs are returned to normal sea water (figs. 204, 207, 212, 213) but the cleavage of the yolk is permanently suppressed.

I do not find, as Loeb did, that the protoplasm in such cases begins to segment into as many cells as there are nuclei preformed; on the other hand there are usually several nuclei (or karyomeres) in each of the cells so formed. Furthermore the cleavage of the protoplasm never occurs during the resting stages of nuclei and centrosomes, but only during periods of mitosis, and there is an evident tendency for the protoplasm to segment around each of the superficially placed centrosomes (fig. 204). Only in cases where these centrosomes are very numerous or where they lie some distance from the surface are there no constrictions in the protoplasm around each astral system as a center (figs. 203, 219).

Loeb believes that the failure of plasma to divide, in hypertonic solutions, is due to the withdrawal of water from the egg. In my experiments hypotonic solutions as well as hypertonic ones cause a suppression of cell division. In the former the egg takes up water, in the latter it loses water, and it does not seem possible that diametrically opposite conditions should produce identical results. Many other conditions also lead to the suppression of cleavage, especially in the yolk,—indeed this is the one abnormality of development which is most readily produced by any change in the environment,—and it shows that in such eggs as those of *Crepidula*, holoblastic cleavage may easily be transformed into meroblastic. A study of the catalogue of these experiments given on pp. 559–576 will reveal the fact that *suppression of cleavage, especially in the yolk, may be caused by pressure, electric current, cold, ether, reduced oxygen tension, increased carbonic acid, diluted sea water and concentrated sea water. If cleavage is the result of some simple physical process, such results are difficult to understand, but if it be the result of more complex physiological processes, such as contractility or movements on the part of the plasma, then it is possible to understand how anything which weakens or disturbs this function of the plasma may cause stoppage of cell division.* When eggs are returned to normal sea water after having been subjected to hypertonic solutions the various cell constituents, if they recover their power of division, return to it in the inverse order of their suppression in the solutions, activity reappearing first in the centrosomes, then in the nuclei, then in the protoplasm and finally in the yolk.

3. *Suppression of all Forms of Division without Stopping Nuclear Growth.*

(Figs. 171–173, 176.)

In solutions of 1 per cent. KCl, 2 per cent. NaCl, or 4 per cent. $MgCl_2$ in sea water, which are allowed to act for approximately 9 hrs., or in stronger

solutions which are allowed to act for a correspondingly shorter time, all forms of division are permanently suppressed, so that when eggs subjected to such solutions are returned to normal sea water they never show any signs of division either of cytoplasm, nucleus or centrosome. Nevertheless such eggs may remain alive for several days and their nuclear vesicles may grow to enormous size. In such eggs centrosomes and spheres are usually entirely lacking, or at best are small and indistinct; the chromatin, which is always contracted into a nucleolus-like mass within the nuclear vesicle while the eggs are in these solutions, remains permanently in this condition when the eggs are returned to normal sea water, and never again assumes a reticular form, nor gives rise to chromosomes. I attribute the complete cessation of divisional activity to this inability of centrosomes and nuclei to recover their normal forms and functions.

The nuclear vesicles grow to a great size and in this condition they have all the appearances of germinal vesicles of immature eggs. One cannot fail to be impressed with the resemblance of such cells to immature egg cells, not merely in their form but also in the loss of the power of division, their low metabolic activity, and their ability to remain alive almost indefinitely while in this condition. It would be worth while to treat such eggs with some of the various reagents by which it has been found possible to stimulate immature eggs to form polar bodies, in order to see whether divisional activity might again be revived in them.

In addition to the limiting action of hypertonic solutions on the division of the cell body, the nucleus, and the centrosome, other striking results are produced which will now be described.

4. *Shrinkage of Plasma, Nuclei and Mitotic Figures in Hypertonic Solutions.*

The first effect of hypertonic solutions on the eggs of *Crepidula* is to cause a shrinkage, or contraction, of the plasma, nuclei, and mitotic figures, probably accompanied by the loss of a small amount of water from the egg, as Loeb has maintained. However since the diameter of the egg as a whole decreases very little, the loss of water must be slight. Indeed this shrinkage shows itself not so much in the decrease in size of the entire egg as in the more complete segregation of the plasma from the yolk, on the one hand, and from the more fluid inclusions (oil, water, etc.) on the other. This is accomplished, apparently, by the withdrawal of outlying portions and radiations of plasma into a central mass immediately surrounding the nucleus, while at the same time the yolk and more fluid inclusions are forced to the periphery of this mass. In this way a segregation of these three constituents of the egg is accomplished, similar in the nature of the substances segregated, but not in their orientation, to the segregation accomplished by centrifugal force.

In wholly similar manner the shrinkage of the nucleus as a whole is relatively slight, whereas the segregation of chromatin from achromatin is the most distinctive action of hypertonic solutions on resting nuclei. The chromatin within

the nuclear vesicle is clumped or condensed into a single central mass, or into a dense reticulum of very coarse threads; the surrounding achromatin[1] is left free from granules of chromatin and yet it stains like oxychromatin and is identical with the material which I have elsewhere (1902) called "chromatic nuclear sap."

During mitosis this chromatic sap together with hyaloplasm from the cell body constitutes the archiplasm, which fills the area around the centrosomes, radiates along the astral fibers and forms the interfilar substance of the spindle as I have shown in the work just referred to (1902, p. 49). In hypertonic solutions this archiplasm is withdrawn from the astral radiations into the spindle and the area immediately surrounding it (figs. 189, 190). At the same time the entire spindle figure shrinks in size, the chromosomes are usually clumped together, and the spindle fibers may disappear. Eggs which have been returned from hypertonic solutions to normal sea water lose all appearance of shrinkage within a short time (Exps. 973, 977). Konopacki found that such eggs assume a normal appearance within five minutes after their return to normal sea water.

5. *Formation of Cytasters and Polyasters.*

(Figs. 183–190, 199, 203, 204, 206, 207, 213, 219, 220.)

All investigators who have made a cytological study of the effects of hypertonic solutions on eggs have emphasized the fact that the appearance of cytasters constitutes one of the most characteristic results.[2] Morgan (1899) in particular holds that the presence of cytasters (his astrospheres) rather than the suppression of cell division is the most distinctive effect of hypertonic solutions. Wilson (1901) believes that these cytasters may contain centrosomes, become the poles of spindles, and in every other way behave as veritable centers of mitotic division. Admittedly the evidence in favor of this view is not entirely conclusive, and Petrunkewitsch (1904) maintains that there is a fundamental distinction in these regards between "nuclear asters" and "cytasters," with which opinion I agree.

The exact method of the origin of cytasters has not been traced, except by Konopacki (1911), who finds that they arise along the astral radiations and from the material of which these radiations are composed. He finds that they appear most frequently before cleavage, more rarely in the 2-cell stage, and never in later stages. My own observations entirely confirm those of Konopacki in these respects. In 1902 I showed that cytasters never form when the germinal vesicle is intact, and that they form from archiplasm which is derived in part from escaped achromatin of the nucleus.

[1] The term "achromatin" as originally introduced by Flemming (1882) includes all materials of the nucleus other than chromatin. As thus used it is a generic term including oxychromatin, linin, nuclear sap, and possibly still other substances. Since these different substances are not distinguishable at every stage of the nuclear cycle it is convenient to have a generic term which will include any or all of them and it is for this reason that the term "achromatin" is used.

[2] Not infrequently cytasters appear in eggs under normal conditions, especially during the period between fertilization and the first-cleavage (cf. Exp. 996 (1), control on effects of electric current).

In the withdrawal of the archiplasm of the astral radiations into the spindle, certain portions may be left isolated along the rays, especially if these rays are very long, as is the case in the 1-cell and 2-cell stages. These isolated portions of archiplasm become the centers of new radiations, thus forming cytasters. These cytasters sometimes contain deeply staining granules, which have the the appearance of centrioles, and which may be derived from the axial filament of the radiation, but so far as my observations go, they never become the poles of spindles nor undergo division. On the other hand cytasters frequently fuse together, as Mead (1898) and Morgan (1899) have determined, whereas nuclear asters often divide, but rarely fuse. If very many cytasters are present in a cell the astral systems at the poles of the spindles are smaller than usual, because the archiplasm which largely composed these systems is distributed to many centers; this fact has been observed and commented upon by both Morgan and Wilson. Cytasters appear best developed during periods of mitosis and if a long resting period follows, the place of the cytaster is taken by a faintly staining vesicle of archiplasm or achromatin (figs. 186–188, 191, 192). The substance thus isolated forms a delicate membrane and gives rise to a small "nucleus without chromatin" (R. Hertwig). I have not determined positively that such an achromatic vesicle can give rise to another astral system at the next mitosis but such seems to be the case. Where cytasters occur during interkinesis it is invariably found that a large amount of archiplasm is distributed through the cell, as for example after the maturation divisions and before the first cleavage. It is well known that a great amount of achromatin escapes from the germinal vesicle at the time of the first maturation division, and this achromatin is widely distributed through the egg along the astral radiations. When the germ nuclei have grown large and have therefore absorbed a great deal of achromatin no cytasters are formed, especially in the vicinity of the nuclei, but when the nuclei are small and much achromatin is still scattered through the cell, cytasters may appear. *The cytasters are therefore, in my opinion, isolated portions of archiplasm, derived in large part from escaped achromatin, which take the aster form during mitosis and the vesicular form during resting periods* (figs. 183–193).

In addition to the presence of cytasters in eggs exposed to hypertonic seawater, several nuclear asters may be present and since all asters within the same cell are in divisional activity at the same time, multipolar mitoses thus arise (figs. 203, 219, *et al*). These polyasters are found only in cells in which cleavage has been temporarily suppressed while centrosomal division proceeded, or in cells in which cleavage has been permanently suppressed, while the centrosomes have continued to divide after the eggs were returned to normal sea water; in the latter case their number is generally proportional to the length of time the eggs have been in normal sea water. These polyasters pass through periods of division, alternating with periods of rest, and there is reason to believe that they have arisen by regular division from an original single centrosome and aster in each cell; such division would give rise to, first amphiasters, then tetras-

ters, and finally polyasters in cases where the division of the cell body is inhibited, while that of the centrosome proceeds. *I have been unable to find any evidence whatever that centrosomes arise de novo in the eggs of Crepidula, or that cytasters become the centers of mitotic nuclear division.*

It is a striking fact, and one not easily harmonized with the hypothesis that centrosomes may arise *de novo*, or from the fragmentation of a single centrosome into many, that the number of nuclear centrosomes in eggs in which cleavage was suppressed by hypertonic sea water is approximately proportional to the length of time during which the eggs have remained in normal sea water following exposure to the hypertonic solutions. The maximum number of cytasters is found while eggs are still in the hypertonic solution (figs. 183, 189, 190) whereas in such eggs the nuclear asters are present in normal numbers. Only after such eggs have been returned to normal sea water for several hours during which time centrosomal division has been progressing while cell division remains suppressed do nuclear asters become numerous (figs. 203, 207, 219, *et seq.*). The only apparent exceptions to this rule are found in those cases in which the hypertonic solution (or other injurious condition) was not strong enough to stop centrosomal division, although strong enough to suppress cell division, and these exceptions are only apparent and really support the proposition that nuclear asters and centrosomes arise by the division of preexisting asters and centrosomes. Further evidence that the polyasters which occur in the eggs of *Crepidula* are not derived from cytasters may be found in the fact that polyasters may be found at any stage of the cleavage, whereas according to Konopacki and myself, cytasters are rarely found later than the 2-cell stage.

6. *Origin of the Cleavage Centrosomes.*

(Plate LV.)

All of the eggs represented in plate LV were placed in 1 per cent. NaCl in sea water for 4 hrs. and were then fixed at once. In all of these figures both polar bodies had been formed and the sperm had entered the egg before the experiment began. The effect of the hypertonic sea water has been to delay the movement of the sperm nucleus toward the egg nucleus and to stimulate the formation of an amphiaster in connection with the egg nucleus. The view was first expressed by Boveri (1892), and has been widely accepted by other investigators, that the egg centrosome is a decadent structure, which ultimately undergoes degeneration, while the two cleavage centrosomes arise in connection with the spermatozoon. In cases of normal or artificial parthenogenesis it is supposed that the cleavage centrosomes are derived from the egg centrosome, which in such cases becomes active, or they are new formations. My work on the normal processes of maturation and fertilization in *Crepidula* (Conklin, 1902) led me to the conclusion that *one of the cleavage centrosomes is formed in connection with each of the germ nuclei*, though it was impossible to say that they came from the

egg and sperm centrosomes, since the latter had disappeared as such, before the first appearance of the cleavage centrosomes.

In the eggs represented in figs. 159–163 various stages in the division of the egg centrosome and in the formation of the egg amphiaster are shown. That this centrosome and amphiaster actually belong to the egg and not to the sperm is beyond doubt, the sperm nucleus being so far removed from the egg nucleus and so isolated from it by intervening yolk that there is no possibility of confusing the two. In the figures mentioned the sperm centrosome and aster are not visible, if present. In fig. 164 two spindles are present in the egg, one of which is certainly the egg spindle and the other the sperm spindle; the same is true of fig. 168, in which the two spindles are entirely distinct. In all other figures on plate LV, viz., figs. 165–167, 169–170, the two spindles have not remained distinct, but their poles have interfered, thus giving rise to tetrasters.

I have discussed these figures elsewhere (Conklin, 1904) and need not comment on them here further than to call attention to the fact that the origin of amphiasters in connection with each of the germ nuclei, when the latter are kept apart, indicates that the egg centrosome does in this case persist, and that it may be stimulated to divide and form an amphiaster by methods which have elsewhere been employed to produce artificial parthenogenesis. In all probability this is just what occurs in every case of normal or artificial parthenogenesis; the cleavage centrosomes being derived from the egg centrosome, and not being formed *de novo*.

Kostanecki (1906, pp. 386–394) has commented at some length on my conclusions as to the origin of the cleavage centrosomes in *Crepidula* under normal and artificial conditions. So far as the origin of these centrosomes under normal conditions is concerned I again admit, as I have done in previous papers (1901, 1902, 1904), that the evidence is not conclusive that they come, one from the egg centrosome and the other from the sperm centrosome, since both of the centrosomes have disappeared as such before the cleavage centrosomes appear. On the other hand the persistence of the egg and sperm spheres until the union of the germ nuclei, the fusion of the spheres and the appearance of a cleavage centrosome in connection with each of the germ nuclei, the absence at all stages of the normal process of an amphiaster in connection with either the egg or the sperm nucleus—these incontrovertible facts do not afford evidence for the view that the cleavage centrosomes always come from the sperm centrosome. I freely admit that in many instances such an origin may be clearly traced. In certain ascidians which I have studied (Conklin, 1905) there are no centrosomes in either the first or second maturation divisions, and none at any stage in connection with the egg nucleus; while the origin and division of the sperm centrosome and its transformation into the sperm amphiaster and into the first cleavage spindle can be determined with the greatest certainty. There is here a great difference between *Ciona* or *Cynthia* and *Crepidula* or any other gasteropod.

These differences may be summarized in the following tabular statement:

CREPIDULA.	CIONA.
Egg with abundant yolk.	Egg with little yolk.
Large centrosomes and asters in both maturation spindles.	No centrosomes or asters in either maturation spindle.
Egg centrosome remains evident for some time and then disappears in egg sphere.	No egg centrosome at any stage.
Egg aster gives rise to large sphere which persists till germ nuclei conjugate.	No egg aster or sphere at any stage.
Sperm nucleus moves very slowly toward egg nucleus; first cleavage is about 6 hrs. after fertilization.	Sperm nucleus and egg nucleus move rapidly toward posterior pole; first cleavage is about 40 min. after fertilization.
Sperm nucleus, centrosome and aster lie for 3 or 4 hrs. in compact yolk and remain small.	Sperm nucleus, centrosome and aster lie in cytoplasm from the first and grow rapidly.
Sperm centrosome does not divide but disappears in sperm sphere which persists till germ nuclei conjugate.	Sperm centrosome divides giving rise to sperm amphiaster.
Egg and sperm spheres fuse and disintegrate when germ nuclei conjugate, and one cleavage centrosome appears close to each nucleus, and the cleavage spindle forms between them.	Sperm amphiaster becomes the central spindle of the first cleavage.

When to these differences in the normal phenomena of fertilization is added the fact that hypertonic sea water may cause a perfect spindle to form in connection with each of the germ nuclei in *Crepidula* but that no means has yet been found, though many have been tried, to cause a spindle to form in connection with the egg nucleus of ascidians, the extent of the differences between the eggs of these two types will be appreciated.

The view that this difference ceases when we come to the actual origin of the cleavage centrosomes is based not upon actual evidence but upon the conviction that a single, uniform origin of these centrosomes must be expected,—that in spite of all other differences there must be uniformity in this regard. And yet nothing is more certain than that the cleavage centrosomes do not have a uniform origin in all cases, that while, in many instances they arise in connection with the sperm nucleus, in all cases of normal or artificial parthenogenesis they cannot have such an origin. There is not therefore a single, uniform origin of these centrosomes in all animals, and consequently there is no antecedent probability that the differences observable between *Crepidula* and *Ciona*, for example, with respect to other phenomena of maturation and fertilization do not also extend to the origin of the cleavage centrosomes. If it were certain that the cleavage centrosomes invariably come from the sperm centrosome in fertilized eggs, and invariably from some other source in parthenogenetic eggs,

there would be ground for establishing a fundamental distinction between these two types of eggs; but *the fact that any one of many slight changes in the environment may cause a non-parthenogenetic egg to become parthenogenetic indicates that no such distinction can be of a very fundamental nature, and it also indicates that there is no inherent improbability in the view that cleavage centrosomes may in some cases arise from the egg centrosome as well as from the sperm centrosome.*

Kostanecki believes that my views are based upon a too narrow consideration of the case of *Crepidula*. I, on the other hand, believe that the view that the cleavage centrosomes always come from the sperm centrosome is based upon a too narrow view of the conditions in certain fertilized eggs, with a corresponding neglect of the conditions in normally and artificially parthenogenetic eggs.[1] I cannot better sum up my present views on this subject than by quoting the conclusions reached in a former paper (1902, p. 30), to which I still adhere: "When one looks at the problem of fertilization from a general point of view, when one considers the universality of sexual reproduction, when one reflects upon the multitudes of exquisite adaptations which exist for securing the union of egg and sperm, he will be loath to believe that the essential feature in fertilization is the addition of a centrosome to the egg cell, or the supplying of a stimulus to its development, which is not needed in all cases and which can as well be supplied by changes in density, salinity, temperature, etc., as by the entrance of a spermatozoon."

7. *Irregularities in the Movements of Chromosomes.*

In the separation of daughter chromosomes great irregularities appear; "lagging" chromosomes are almost always present and in many cases the chromosomes are scattered along the whole length of the spindle (figs. 211–222). As a result of these irregularities of movement on the part of the chromosomes they are not distributed equally to the two poles of the spindle and they do not come together at the poles to form a single nuclear vesicle, but they become associated in varying numbers to form vesicles, or karyomeres, of different sizes, depending upon the number of chromosomes which enter into them, while if scattered along the whole length of the spindle, the chromosomes may form a continuous chromatic connection between daughter nuclei, thus giving rise to conditions which are falsely suggestive of amitosis. The karyomeres may be few

[1] Wilson (1897) demonstrated that in echinid eggs the entire middle piece of the spermatozoon was not involved in the formation of the sperm centrosome. Meves (1911) has shown that no portion of the middle piece is necessary for the formation of the sperm centrosome. F. R. Lillie (1912) in a recent and most important paper has shown that in *Nereis* both middle piece and tail of the spermatozoon remain outside the egg and that the sperm centrosome arises from the base of the sperm head. Furthermore portions of the elongated sperm nucleus may be stripped off from the egg along with the jelly membrane, by centrifugal force, and in such cases the sperm centrosome always arises from the basal end of that portion of the nucleus which enters the egg, and is proportional in size to the quantity of nuclear material which thus enters. He concludes "that the centrosome and aster owe their existence to an interaction between nucleus and cytoplasm and not to any third element." The view that the cleavage centrosome can come only from the centrosome of the spermatozoon is evidently losing ground.

or many in number, and they may be entirely distinct or may be partially united, thus giving rise to nuclear forms which might be interpreted as nuclear constriction or budding (figs. 175, 177, 194–196, 198, 200–202, 205–208). Indeed they are so interpreted by Konopacki, but in *Crepidula* a careful study of the karyomeres with regard to their stages in the nuclear cycle shows that the unions are more complete in the later than in the earlier stages, thus proving that they are fusing rather than separating. However, where the karyomeres are quite distinct they may remain separate in all the cells through several cell generations (figs. 205–208); evidently the union of the karyomeres into a single vesicle can take place only when they are in intimate contact.

In all my preparations the nuclear vesicles are much more numerous than the nuclear asters. If both nuclei and asters multiplied by constriction or fragmentation this condition would be difficult of explanation, but if the nuclear asters and centrosomes arise by bipartition, while the karyomeres come from scattered chromosomes, the explanation of this condition is obvious. The centrosomes are unit structures, the nuclei are compound, being composed of many chromosomes or chromosomal vesicles; while each centrosome becomes the center of an aster, each chromosome does not usually form a separate nuclear vesicle, though it is capable of doing so, but unites with other chromosomes to form one or many nuclear vesicles.

Considering the fact that these irregularities in the movements of chromosomes are among the most common forms of abnormal cell division whatever the disturbing factor may be, the question arises whether many of the cases of amitosis in normal developmental processes, which have been recently described, may not be really cases of the formation of separate karyomeres, and their subsequent fusion, such as occurs in these experiments. I refrain from commenting here on this subject further than to suggest that many of the figures given by recent writers on this subject bear a striking resemblance to these karyomeres of *Crepidula*. In the latter case, however, the actual lineage of these karyomeres may be determined with great certainty and it can be affirmed that they arise from irregularities in the separation of chromosomes in mitotic division.

The size of these karyomeres is, within the same cell, proportional to the number of chromosomes which enter into them, as Boveri (1905) found to be the case in echinoderm eggs. However in cells which differ in their quantity of cytoplasm, nuclei and karyomeres may differ in size, though they contain the same number of chromosomes, since the ultimate size of a nucleus depends not merely upon the number of chromosomes which it contains but also upon the quantity of cytoplasm in which it lies (Conklin, 1912).

8. *Segregation of Chromatin and Achromatin.*

Attention has already been called to the fact that isolated portions of achromatin may assume the form of cytasters during periods of mitosis and of vesicles with a definite membrane during periods of rest. The former condition

is illustrated in figs. 183, 185, 189, 190; the latter in figs. 184, 186, 188, 191. When eggs are left for a long time in salt solutions, the chromosomes of daughter nuclei are unable to absorb achromatin and to become vesicular but remain small, densely chromatic masses, and in such cases the achromatin forms vesicles which may lie close to the chromatin (figs. 184–188) or may surround the chromatin while remaining distinct from it (figs. 178–183). Indeed the entire achromatic portion of the mitotic figure, with the exception of the centrosomes and spheres, which always remain distinct, may be inclosed in the achromatic vesicle (figs. 178–182). Apparently the achromatic vesicle does not form until the telophase of division, and even then it may form in the place of one spindle and not of another (fig. 180), or in one of two daughter cells and not in the other (fig. 181). This achromatic vesicle has a very thin membrane and the whole vesicle is elongated in the direction of the spindle axis. Since this achromatic vesicle conforms to the outlines of the spindle in telophase, it is conical in shape, its base being turned toward the centrosome and its apex toward the *Zwischenkörper*. The appearance of two such conical vesicles in daughter cells with their apices turned toward each other, is strongly suggestive of the constriction and amitotic division of the achromatin. It resembles still more those forms of nuclear division in which the chromatin divides mitotically, but in which the nuclear membrane persists throughout the process, and it and the achromatin divide by constriction. However in this case there can be no doubt that the nuclear membrane actually disappears in mitosis while the membrane which forms around the achromatin is a new structure called forth by the action of the salt solutions. Konopacki has observed and figured in echinoderm eggs subjected to hypertonic solutions conditions similar to those just described for *Crepidula*. He finds, as I do also, that this membrane is not of the usual type, but is much thinner, nevertheless he seems to regard these forms as due to an elongation and constriction of the nuclear vesicle, in which, presumably, the membrane persists throughout division. Such is plainly not the case in my experiments, where this achromatic vesicle may be present in one daughter cell and not in the other, as in fig. 181. Kostanecki (1904) has described a form of "intranuclear karyokinesis" in eggs of *Mactra* subjected to hypertonic solutions in which a spireme, chromosomes, and a bipolar spindle form within the nuclear vesicle; the chromosomes separate and move to the two poles and the vesicle and central spindle then disappear while the chromosomes form daughter nuclei. During this whole process faint radiations may be present in the plasma, but there are no centrosomes or astral systems. I have not observed this form of mitosis in *Crepidula*.

These results throw light on the constitution of the nuclear membrane. By various writers this membrane has been held to be composed of an outer plasmatic or achromatic layer and an inner nuclear or chromatic one. My own observations (1902) led me to adopt the view of Van Beneden (1887) that this membrane was formed from the peripheral walls of the chromosomal vesicles, which

fuse to form the nuclear vesicle. Its outer achromatic layer was supposed to be derived from the sheath of linin covering the chromosomes themselves. In the light of the observations here recorded it is evident that *an achromatic membrane may be formed wholly independently of the chromosomes. This membrane is thinner than the usual nuclear membrane and it lacks a chromatic layer. It is probably a precipitation membrane.*

Evidently the growth of the nuclear vesicle in hypertonic solutions and its ability to absorb achromatin is dependent to a certain extent upon the stage of development of the nucleus, and more particularly of the chromatic membrane. In figs. 171 and 172 the egg nucleus has remained small and its vesicle contains only chromosomes and a very clear fluid, in spite of the fact that this nucleus lies in the cytoplasmic area, the nuclear membrane is also thin and achromatic; the sperm nucleus, on the other hand, has grown to a great size and contains most of the achromatin (chromatic sap) which was previously scattered through the cell. If the egg nucleus has passed a certain stage in its development from the chromosomes which were left in the egg after maturation, it also grows, as the sperm nucleus does, and if the resting period is prolonged both germ nuclei may become very large (fig. 173). If any of the achromatin is scattered through the egg as cytasters or vesicles (fig. 174) the germ nuclei are correspondingly smaller. These results indicate that the substance absorbed by the nucleus from the cell is a specific substance and not merely the general material of the cell body.

In a previous paper (1912) I have shown that young nuclei grow by the absorption of material from the middle zone of centrifuged eggs, and not from the lighter or heavier zones. The growth and ultimate size of nuclei was found to be proportional to the abundance of the material of this middle zone, and to the length of time during which it was being absorbed by the nucleus. From the present work it is apparent that *the substance which contributes most largely to the growth of the nuclear vesicle is achromatin which escaped from the nuclear vesicle at some previous mitosis. This achromatin, or chromatic sap, forms the interfilar substance of the spindle and asters and radiates through the cell along the astral fibers; it gives rise to cytasters and achromatic vesicles; it contributes to the growth of the daughter nuclei; it is probable that chromatin grows at its expense and that it in turn receives certain substances from the chromatin; and finally it seems likely that it is this achromatin which establishes connections between the chromatin and the cell body, through which connections the influence of the chromosomes on the differentiations of the cell body are exerted.*

9. *Abnormal Mitosis and Amitosis.*

There are many abnormal mitoses in these experiments which are superficially like amitoses. Among these may be recognized the following classes: (1) Those which are due to irregular separation of daughter chromosomes which are scattered along the length of the spindle; in these forms a chromatic connection is left between daughter nuclei which is at first glance strongly suggestive

of amitosis but a careful study of these nuclei which are connected by a chromatin thread shows that in *Crepidula* at least, they are invariably formed in the manner stated (figs. 141, 143, 144, 152–154, 215, 222). It is highly probable that the number of chromosomes which go into two such daughter nuclei is not the same. Such eggs do not give rise to normal embryos or larvae though subsequent cleavages may go on in a manner which is approximately typical. (2) Apparent amitosis due to the constriction of the achromatin and of the achromatic vesicle following the mitotic division of the chromatin. It is evident that this is merely a variation of the condition found in certain protozoa in which the nuclear membrane does not disappear, and an intranuclear spindle is formed. In *Crepidula*, however, the membrane does disappear, but if the daughter chromosomes are prevented from absorbing achromatin, an achromatic membrane may form around the achromatin of the spindle area, and in the later stages of mitosis such figures may look like amitotically dividing nuclei (figs. 178–182). (3) Apparent amitosis really due to the fusion of karyomeres. Many such cases are shown in my figures. Karyomeres are formed whenever daughter chromosomes are slightly separated into groups; this is especially the case when polyasters are present. These karyomeres may be very numerous, but those which are in contact fuse together during the resting period, so that they become progressively larger and less numerous in later stages of the division cycle (figs. 121, 124, 136, 145–150, 175, 177, 192–223).

Much interest has been shown of late in the question whether amitosis occurs in germinal or embryonic cells, since if it does it thereby weakens if it does not destroy the belief in the chromosomes as the sole "bearers of heredity." I hold no brief for this doctrine and have repeatedly urged (1893, 1899, 1905, 1908, etc.) its too narrow outlook on the activities of the cell as a whole. It seems to me incredible that this most general of all cell functions, which includes differentiation, metabolism and reproduction should be the property of only a single cell constituent,—the chromosomes. I have therefore approached this problem without any preconceived bias as to the theoretical necessity of believing in mitosis in all divisions of generative or embryonic cells, and have not been consciously warped by either the *odium mitoticum* or the *odium amitoticum*.

An excellent review of this whole subject is given in Wilson's book, *The Cell* (1900), and in Godlewski's (1909) notable memoir on the inheritance problem, and I shall confine my attention to a consideration of some of the more important recent contributions on amitosis.

After the discoveries which showed that mitosis was the usual form of nuclear division, most of the workers who studied amitosis found it limited to cells already fully differentiated and usually decadent. In recent years a new interest has been given to the problem by the work of Child (1907[1], 1907[2], 1910, etc.), Hargitt (1900, 1904), Patterson (1908), Glaser (1908), Gurwitsch (1905), Gerassimoff (1892), Nathansohn (1900), Foot and Strobell (1911), *et al.*, all of whom maintain that amitosis may occur as a normal process in germinal and embryonic cells.

On the other hand this view is contested by Boveri (1907) and Strasburger (1908) on general grounds and is not confirmed by the observations of Richards (1909, 1911), nor by the experiments of Häcker (1900) and Schiller (1909) on *Cyclops* eggs subjected to ether, nor by the experiments of Němec (1903), who repeated the work of Wasielewski (1902, 1903) on root tips of *Vicia* subjected to chloral hydrate, and reached the conclusion that the supposed amitoses have arisen through the transformation of normal mitotic figures.

On the other hand R. Hertwig (1898), Herbst (1909), Godlewski (1909), Konopacki (1911), Lang (1901), Calkins (1901) hold that there is no principal distinction between mitosis and amitosis and that they may both occur without interference with normal processes of reproduction and differentiation.

So far as concerns many of the observations and experiments named it must be admitted that they are not critically conclusive; either it is not shown beyond question that the divisions are genuine amitoses, or it has not been proved that the cells are normally differentiating embryonic cells. In particular most if not all of the figures given by Child, Hargitt, Patterson, Glaser and Gurwitsch may be duplicated by figures in which it is known that division is by mitosis. The wholly or partially distinct nuclear vesicles may be fusing rather than separating, and such is undoubtedly the case in my experiments. Godlewski recognizes the difficulty of distinguishing the fusions of karyomeres from direct nuclear divisions, but in my experiments this distinction can be made with certainty, not only because every stage in the formation and fusion of karyomeres may be seen in the division of a particular cell, but also because the relative stages of karyomeres in the division cycle can be distinguished by the character of the chromatin, and the fusions are always more complete in the later than in the earlier stages.

A further matter to which I believe attention has not hitherto been called is that the scattering of chromosomes along the length of the spindle leads to the formation of chromatic connections between daughter nuclei in the resting stage. Such chromatic connections have hitherto been accepted as undoubted proof of amitosis, and Godlewski in his review, after dismissing many other more or less doubtful cases, bases his conclusion that amitosis may occur in normally differentiating tissue upon the undoubted cases given in the works of Gurwitsch and Nathansohn. I am unable to speak from personal experience of the results obtained by the last named investigator in his experiments on *Spirogyra*, but the figure given by Gurwitsch of a mitotic division following an amitotic one in a blastomere of a centrifuged Triton egg, cannot go unquestioned. The chromatic connection between the two nuclei shown, is no evidence of amitosis, but rather of the scattering of chromosomes along the spindle, at the previous division of these cells. I have repeatedly observed such scattered chromosomes and the resulting chromatic connections between daughter nuclei, in practically all of my experiments, though they are figured chiefly in eggs subjected to diluted sea water; they also occur in centrifuged eggs, as I shall show in a later paper.

Of the occurrence of amitosis in protozoa I shall not here treat; the conditions in these forms are peculiar, and the form of nuclear division may also be peculiar, but it seems to me that there is as yet no satisfactory evidence that amitosis ever occurs in normally differentiating cells of the metazoa. Godlewski concludes (p. 120): "Dass aus der bisherigen Literatur sich kein einzige Aufgabe anführen lässt, durch welche ganz positiv bewiesen wurde, dass die Amitose der Karyokinese nicht gleichwertig sein konnte." On the other hand, I think it may be affirmed with equal emphasis that *there are numerous evidences against the view that amitosis can take the place of mitosis in normally developing cells of the metazoa, and not a single entirely positive one in favor of that view.*

XI. Conclusions and Summary.

A. Observations and Experiments.

1. In the eggs of *Crepidula plana* abnormal cleavages and mitoses are relatively rare in the conditions found in nature; those which occur are due, probably, to pressure, diluted sea water, or increased temperature. (Figs. 1–29; pp. 505–507.)

2. The cleavage of isolated blastomeres is strictly partial; the only modifications as compared with entire eggs being due to the rounding of the isolated cells. All the early cleavages are differential (morphogenetic); non-differential cleavages appear only after the formation of ectomeres, mesomeres and entomeres, and in certain subdivisions of these cells. So far as the cleavage stages are concerned the prospective significance and the prospective potency of early blastomeres are identical. (Figs. 30–44; pp. 508–510.)

3. In eggs under pressure lobes may be formed, usually opposite the poles of the spindles; spindle axes and cleavage planes may be turned out of their normal positions, so that the blastomeres formed lie in one plane and bear totally atypical relations to one another and to the axes of the unsegmented egg; if the pressure is in the direction of the spindle axis, but is not sufficient to turn the spindle out of that axis, it may lead to suppression of cell division. In all cases the prospective significance and the prospective potency of such blastomeres, during the cleavage stages at least, is determined by the relations of the cleavage planes to the axes of the unsegmented egg. All cells formed by meridional cleavages give rise to three separate ectomeres at the animal pole, and to one entomere at the vegetal pole; all cells formed by equatorial or latitudinal cleavages are ectomeres at the animal pole, entomeres at the vegetal pole; mesomeres arise only from cells lying between these poles on the posterior side of the egg. There is, presumably, a stratification of the hyaloplasm, or "ground substance," of the unsegmented egg, wholly apart from the yolk or other inclusions, which determines the morphogenetic trend of each blastomere. (Figs. 45–67, 76, 77; pp. 510–518.)

4. When eggs are subjected to a weak electric current, spindles, nuclei and cytoplasm may be displaced as if by pressure and subsequently formed blasto-

meres may be abnormal in position. With a stronger current (or more perfect penetration) chromatin and chromosomes may be clumped into masses or even completely dissolved, while the spindles and asters may be preserved in some cases or dissolved in others. With a stronger current (or more perfect penetration) cytoplasm and yolk may be stratified and nuclei and spindles displaced by convection currents. There is no indication that the poles of spindles bear charges differing in sign from the chromosomes, or that spindle fibers or astral rays represent lines of force in an electric field, or that the movements of chromosomes into or out of the equatorial plate are caused by electric charges carried by the chromosomes and centrosomes. (Figs. 68–93; pp. 518–524.)

5. Increase of temperature from 10° to 16° above that of normal sea water, if only for ¼ hour, leads to profound modifications of cell division. The most general effects are: (1) Reduction of surface tension, with consequent irregularities in the contour of eggs and changes in the type of cleavage. (2) Withdrawal of astral rays and segregation of archiplasm in the spindle area and along the walls between cells. (3) Great changes in the orientation and structure of mitotic figures; loss of spindle fibers and centrosomes; scattering of chromosomes and formation of numerous karyomeres. (Figs. 94–99; pp. 524–525.)

In eggs kept near the freezing point all divisional phenomena are soon stopped; after 14–16 hrs. on ice the centrospheres, during resting stages, become bounded by a definite membrane and look like faintly-staining nuclei; after 40 hrs. on ice centrospheres break up into coarse, chromatic granules, which may be homologous with mitochondria. (Figs. 100–105; pp. 525–526.)

6. In 1 per cent. and 2 per cent. solutions of ether in sea water cell division proceeds with few modifications; in 3 per cent. solutions all karyokinetic and cytokinetic movements cease and cell division is suppressed, archiplasm is withdrawn into the central portion of the aster and into division walls between cells, chromatin is clumped in resting nuclei, chromosomes are scattered along the spindles, and spindle fibers are indistinct or absent. (Pp. 526–527.)

7. In water in which the oxygen tension is reduced by boiling, or by running a current of hydrogen through it, cell division is greatly retarded or completely stopped, but cleavages are normal in type as far as they go; resting nuclei and nucleoli become larger than usual during the long interkineses, and the chromatin is clumped into a nucleolus-like mass; centrospheres lose their definite outlines and consist of coarse, widely-scattered granules; spindle fibers, chromosomes and centrosomes remain distinct, but astral radiations disappear, the archiplasm being drawn into the central figure of the amphiaster and into the division walls between cells. (Figs. 106–118; pp. 527–529).

8. Addition of CO_2 to sea water causes local reductions in the surface tension of both cells and nuclei, whereby their surfaces become lobulated; also the positions and directions of mitotic figures and of the resulting cell divisions are altered. (Figs. 119–131; pp. 530–532.)

9. Hypotonic sea water may induce polyspermy; isolation of blastomeres;

suppression of cleavage in the yolk, with subsequent formation of polyasters and meroblastic cleavage; scattering of chromosomes, with consequent formation of karyomeres, chromatic connections between daughter nuclei, and amitosis-like figures. (Figs. 132–158; pp. 532–536.)

10. In sea water rendered hypertonic by the addition of KCl, NaCl, or $MgCl_2$, yolk cleavage is suppressed in weaker solutions, protoplasmic cleavage in stronger solutions, nuclear and centrosomal divisions in still stronger solutions; archiplasm, chromatin and mitotic figures shrink, and cytasters are formed from isolated portions of archiplasm of the astral radiations; multiple nuclei and multipolar spindles are due to the suppression of cell division while nuclear and centrosomal divisions proceed; irregular movements of chromosomes lead to the formation of isolated chromosomal vesicles or karyomeres; achromatin may separate from chromatin and form achromatic vesicles with achromatic membranes. (Figs. 159–223; pp. 536–550.)

B. Cleavage and Differentiation.

11. Early stages in development are more easily influenced by environmental changes than are later ones; and stages during kinesis are more susceptible to modification than stages during interkinesis. Almost all persistent alterations of structure occur during cell division, few of those which occur during the resting period are permanent. That this is due primarily to interference with the divisional apparatus rather than to increased permeability of the plasma membrane during division is indicated by the facts (1) that this is true of eggs subjected to pressure and to the electric current as well as to the various solutions used; (2) that weak solutions produce more changes of a permanent character when acting during kinesis, than do much stronger solutions acting during interkinesis; (3) that practically all persistent modifications of structure are those which affect the division of nucleus, centrosome or cell body, whereas great modifications of structure during the resting period quickly disappear when the eggs are returned to normal conditions. The cause of this is evidently to be found in the fact that when chromosomes, centrosomes or differentiated portions of the cell body are once distributed abnormally to the two daughter cells there is no possibility of bringing about a normal redistribution of these structures, owing to the intervention of the division wall. (Pp. 537, 538.)

12. The study of the development of eggs under pressure, and of isolated blastomeres, shows that there is a polar-bilateral organization of the egg, that the early cleavages are typically differential, and that the prospective significance and the prospective potency of the early blastomeres are identical. On the other hand the nuclear divisions are non-differential and the early nuclei totipotent. (Pp. 515–518.)

13. The causes of differential cleavage are to be found in the relations of the spindle axes and the cleavage planes to the organization (differentiation and localization) of the cell substance. The position of the mitotic figure in a cell is

due to several factors: (1) The separation of daughter centrosomes at right angles to the cell axis (the axis passing through centrosome, nucleus and mid-body), and in the plane which separates the gonomeres. (2) Telokinetic movements at the close of each mitosis by which the cell axis undergoes regular changes with respect to the axis of the egg as a whole, the poles of the cell axis always moving toward the animal pole and toward the point where the previous cell constriction began. (3) Local reductions of tension of the cell membrane in certain axes, or increase of tension in other axes, by which an axis of least resistance is established in the cell. (4) Mitotic movements of the cell contents, which begin with the dissolution of the nuclear membrane in the prophase, and which cause the spindle and the surrounding plasma to move into certain definite axes and positions in the cell. Of all of these factors the last named is, during the early cleavages at least, the most important and the most difficult of explanation.

14. Cleavage furrows may be suppressed, without stopping subsequent nuclear and centrosomal divisions, by shaking, pressure, increased temperature, carbonic acid, diluted sea water and concentrated sea water. The first and second cleavages may be suppressed without stopping subsequent cleavages, and in this way a holoblastic type of cleavage may be transformed into a meroblastic type.

(1) When the second cleavage furrow is suppressed subsequent cleavages may go forward in typical manner, provided the two mitotic figures in each blastomere (*AB*, *CD*) remain so far apart that they do not interfere; in this way three quartets of typical ectomeres (1*a*–1*d*, 2*a*–2*d*, 3*a*–3*d*) may be formed and at the fourth cleavage of the blastomere *CD*, two mesentoblasts (4*c*, 4*d*) may form simultaneously, at the 24-cell stage, although in typical development 4*c* is not a mesomere but an entomere, and does not form until the 52-cell stage. Within the same cell two mitoses are always simultaneous, and the resulting daughter cells are similar. The importance of division walls and of the isolation of morphogenetically and chemically different substances in differentiation is thus clearly indicated. (Figs. 123, 129, 157, 158; pp. 531, 532, 534, 538–540.)

(2) If the two mitotic figures in each of the macromeres (*AB*, *CD*) interfere, thus forming triasters or tetrasters, subsequent cleavages are not typical, but each macromere usually forms a single ectomere at each cleavage (1*ab*–1*cd*, 2*ab*–2*cd*, 3*ab*–3*cd*), the ectomeres thus being formed in sets of two instead of in sets of four, as in typical cleavage; of course the nuclei and centrosomes in such ectomeres are usually abnormal; however the fact that, in such cases, three sets of ectomeres may be formed in the typical directions, although the mitotic figures are very abnormal, indicates that typical, differential cleavage is a function of the cytoplasm rather than of the nucleus, and that the positions of the spindles and the locations of the cleavage planes are principally determined by the cytoplasm. (Figs. 123, 128, 137, 138, 211, 212, 213; pp. 531, 535.)

(3) If both first and second cleavages are suppressed multipolar spindles are

present in subsequent mitoses, since the four centrosomes which are typically present in such an egg are sure to interfere; subsequent cleavages lead to the formation of ectomeres which may be formed in sets of one, though the number of sets and the directions of division are difficult to identify; there seems no doubt however of the tendency on the part of the cytoplasm to cut off characteristic ectomeres at the animal pole, even though the nuclei and their divisions may be very atypical. (Figs. 6–8, 120, 121, 134, 135, 145, 148, 197–208; p. 539.)

(4) When either the first or second cleavage furrow is suppressed but the nuclear division goes on, subsequent cleavages may form more or less normally, but the suppressed cleavage is never repeated; if the egg is prevented from completing one of these divisions in its proper sequence it never returns to that division but goes on to the next in order. On the other hand if the third cleavage is turned out of its typical position, so that it becomes meridional instead of latitudinal, the next cleavage in order is morphogenetically the third cleavage, and is typical barring the number of macromeres and micromeres, which is double the typical number; the imposed meridional cleavage is a new one, intercalated between the typical second and third cleavages.

C. Mitosis and Amitosis.

16. In eggs subjected to an electric current there is no evidence that the centrosomes and chromosomes carry electric charges which differ in sign; nor that the mitotic spindle and the astral rays are chains of granules along lines of force in an electric field; nor that the movements of chromosomes into or out of the equatorial plate are due to the attractions or repulsions of electrically charged bodies. On the contrary, when mitotic figures are displaced by convection currents they move as a whole; the spindle fibers are actual threads of more consistent plasma than the surrounding parts, and may undergo bending and stretching without interrupting their continuity; the typical movements of chromosomes into and out of the equatorial plate cannot be explained consistently on the hypothesis that these movements are due to electrical attractions or repulsions between centrosomes and chromosomes. (Pp. 520–524.)

17. As the result of former observations (1902) and present experiments I conclude that the mitotic figure is, in the main, the expression of complicated diffusion phenomena between nucleus, centrosome and cell body; at the beginning of mitosis the "chromatic nuclear sap" (achromatin, archiplasm) escapes from the nuclear vesicle, when the membrane dissolves; it fills the areas around the centrosomes and radiates from these areas and from the entire amphiaster into the cell body; the spindle fibers, although consistent threads, are not preformed structures, but they grow, when first in contact with the chromosomes, in a manner suggestive of the formation of fibrin threads in clotting blood; it is theoretically possible to explain the division of chromosomes and their movement into and out of the equatorial plate by such formation and growth of polar fibers and of interchromosomal (interzonal) fibers; in later stages of mitosis the

spindle fibers are dissolved and taken into the daughter nuclei along with other achromatin (interfilar substance, archiplasm). During later stages of mitosis much of the archiplasm (achromatin) flows from the astral radiations into the central area of the aster and spindle, the daughter chromosomes absorb achromatin, thus becoming vesicular, and these chromosomal vesicles then fuse into several karyomeres and finally into a single nuclear vesicle the wall of which is composed of an outer achromatic membrane and an inner chromatic one, while the chromatin within the membrane takes the form, at different stages, of vesicular walls, reticulum, or granules. (Pp. 520, 542, 543.)

18. The diffusion phenomena between nucleus and cell body may be interrupted or modified by changes in the environment (increased temperature, decreased oxygen tension, ether, diluted sea water, hypertonic sea water) and the material of the astral radiations may be withdrawn into the central area of the amphiaster or into the ectoplasmic layer, or isolated portions of it may be scattered through the cell body. These altered conditions not only prevent diffusion between different substances but they bring about a shrinkage or segregation of these substances, (figs. 94, 96–99, 116, 117, 183, 189, 190). During 1-cell and 2-cell stages when astral radiations are extensive and much achromatin from the germinal vesicle is distributed through the cell, isolated portions of this achromatin form cytasters (figs. 183, 189, 190, 201); these may contain central granules but not true centrosomes; they fuse together but rarely if ever divide, and they never form the poles of nuclear spindles; the size of amphiasters and of nuclei is inversely proportional to the number and size of these cytasters (figs. 171–176, 183–190, etc.), thus indicating that the substance of the cytasters is achromatin withdrawn from the amphiasters and nuclei; if by the continued action of hypertonic solutions cytasters are prevented from fusing with amphiasters and nuclei they form during interkinesis delicate achromatic membranes, thus becoming achromatic vesicles, or "nuclei without chromatin," which may be widely scattered through the cell (figs. 174, 184–191). If the daughter chromosomes are prevented by hypertonic solutions from absorbing achromatin they remain a densely chromatic mass which does not become reticular or granular, and if resting nuclei with their chromatin in the form of a loose reticulum are treated with hypertonic solutions, the chromatic net at once contracts into a dense central mass, suggestive of normal synezesis, which is surrounded by achromatin and achromatic membrane (figs. 178–192). When the typical swelling of the chromosomes, by the absorption of achromatin, is prevented, a delicate achromatic membrane may form around the achromatin of the spindle area, within which the daughter chromosomes form a dense chromatic mass (figs. 178–182); or the achromatic vesicle may form on one side, or some distance from the chromatic mass (figs. 184–188).

19. Cytasters do not, in *Crepidula*, contain true centrosomes nor take part in nuclear division; on the other hand nuclear centrosomes and asters arise, during cleavage at least, by the division of preexisting centrosomes. If cell

division is suppressed while centrosomal division proceeds many centrosomes and asters (polyasters) appear in the cell, but there is no evidence that these arise *de novo* or from cytasters. If the union of germ nuclei, after fertilization, is delayed, the egg centrosome may divide, giving rise to a spindle, and at the same time a spindle may form in connection with the sperm nucleus; if these spindles are far apart they remain independent, if near together they interfere, thus producing tetrasters or triasters (figs. 159–170). The cleavage centrosomes do not invariably arise from the sperm centrosome. (Pp. 542–547.)

20. Many different environmental changes (shaking, pressure, increased temperature, ether, carbonic acid, diluted and concentrated sea water) may cause abnormalities in the separation of daughter chromosomes, and in their fusion to form daughter nuclei. Chromosomes may lag in the equator or be scattered along the length of the spindle, in which case there is left a chromatic connection between daughter cells (figs. 140–144, 152–154); slight separations between chromosomes at the poles of the spindle lead to the formation of separate vesicles or karyomeres (figs. 120, 121, 124, 136, 145–150, 174, 175, 192–223). Such conditions superficially resemble amitoses, but are true mitoses; there is no entirely conclusive evidence that amitosis ever occurs in the origin of the sex cells of metazoa or in the divisions which accompany embryonic differentiation. (Pp. 547–553.)

XII. Catalogue of Experiments on Nuclear and Cell Division in Crepidula.

(Every number represents a separate microscopic slide, and in most instances a separate experiment. The average number of eggs on each slide is not far from one thousand. All preparations were fixed, stained and mounted in balsam, as described on p. 504. All slides are preserved as permanent preparations and have been repeatedly consulted.)

I. Abnormalities Found in Nature.

In addition to occasional abnormalities found among eggs which were prevailingly normal, the following layings were prevailingly abnormal.

Number.	*Stage.*	*Abnormal Conditions.*	*Results.*
942	1-cell to young veliger.	Water warmer and less dense than normal.	Ectodermal cap not overgrowing yolk; exogastrulæ and exolarvæ, figs. 27–29.
943	1-cell to gastrula.	Unknown.	Giant eggs caused by fusion of two or more eggs.
944	1-cell to gastrula.	Unknown.	Eggs vary in diameter from 96 μ to 192 μ, probably due to fragmentation or fusion of eggs under pressure.
945	24-cells to young veliger.	Unknown.	Ectodermal cap not overgrowing yolk; exogastrulæ and exolarvæ.

II. Eggs Shaken and Fragmented.

No.	Stage.	Experiment.	Left in Normal Sea Water.	Results.
855	1–4 cells.	Shaken, then teased out of capsules.	3 hrs.	Many isolated blastomeres and fragmented eggs undergoing partial cleavage. Figs. 32, 33, 35.
856	1–8 cells.	Teased out of capsules.	12 hrs.	8–30 cells, normal development; a few isolated macromeres show partial cleavage.
857	Ca. 80 cells.	Teased out of capsules.	12 hrs.	Early gastrula; normal.
887	1–8 cells.	Shaken.	8 hrs.	20–29 cell stage of normal eggs; many isolated macromeres show partial cleavage.
888	2–4 cells.	Shaken hard.	15 hrs.	Ca. 50 cells, of normal eggs; many isolated macromeres show partial cleavage, others, not dividing, have large nuclei (synkaryonts).
889	2–4 cells.	Shaken hard.	15 hrs.	Similar to preceding.
890	2–4 cells.	Shaken hard.	24 hrs.	Ca. 68 cells in normal eggs; many isolated macromeres and fragmented eggs, some of them showing partial cleavage.
891	2–4 cells.	Shaken hard.	24 hrs.	Similar to preceding.
892	1–8 cells.	Shaken.	25 hrs.	52–70 cells in normal eggs; many partial eggs.
921	1–4 cells.	Shaken.	2 hrs.	1–8 cells; some isolated macromeres and fragmented eggs. Fig. 31.
922	24 cells.	Shaken.	2 hrs.	Ca. 30 cells; some fragmented eggs.
923	1–24 cells.	Shaken.	4 hrs.	1–36 cells; few eggs not destroyed.
924	1–24 cells.	Shaken.	4 hrs.	1–36 cells; egg fragments.
925	1–30 cells.	Shaken.	4 hrs.	Many isolated blastomeres and egg fragments.
926	1–4 cells.	Shaken.	4 hrs.	A few isolated blastomeres and fragmented eggs, spindles out of position in a few cases.
927	1–2 cells.	Shaken.	4 hrs.	Few eggs, showing few changes.
928	1–4 cells.	Shaken.	6 hrs.	1–8 cells; similar to No. 926.
929	4 cells.	Shaken.	6 hrs.	Few eggs, showing few changes.
930	2 cells.	Shaken hard.	12 hrs.	16–24 cells; few eggs left; a few isolated macromeres.
931	1–8 cells.	Shaken and pressed.	2–12 hrs.	1–24 cells; more than 4 macromeres.
957	2–24 cells.	Shaken.	4 hrs.	Many egg fragments and isolated macromeres, the latter undergoing partial cleavage.
958	2–24 cells.	Shaken.	17 hrs.	24–68 cells; many partial eggs, some showing abnormal yolk cleavage. Figs. 40, 43, 47.
959	1–4 cells.	Shaken.	24 hrs.	1–52 cells; many isolated macromeres showing partial cleavage; in some macromeres cleavage suppressed, but many nuclei and asters. Fig. 44.

III. Effects of Pressure.

No.	Stage.	Experiment.	Duration.	Free in Sea Water.	Results.
849	1–2 cells.	Pressed between slides.	4 hrs.	13 hrs.	16–30 cells; many abnormal cleavages, yolk cleavage being increased in some egg and suppressed in others, depending upon the axis of pressure.
850	1–4 cells.	Pressed between slides.	5½ hrs.	6 hrs.	2–24 cells; many cleavages abnormal in size and direction; yolk cleavage may be increased or suppressed.
851	1 cell.	Pressed between slides.	8 hrs.	11 hrs.	20–24 cells; 1, 2, or many macromeres due to suppression or increase of yolk cleavage; polyasters and multiple nuclei.
852	2–4 cells.	Pressed between slides.	13 hrs.	4 hrs.	8–30 cells; similar to preceding; some blastomeres isolated.
853	1–4 cells.	Pressed between cover glasses.	14½ hrs.	3 hrs.	4–30 cells; many normal eggs; abnormal forms like those in No. 852.
854	1–4 cells.	Pressed between cover glasses.	18 hrs.	4 hrs.	1–30 cells; in some eggs all cleavage suppressed; others show abnormal forms similar to preceding.
884	1–2 cells.	Pressed flat under cover.	3½ hrs.	12½ hrs.	1–30 cells; many isolated blastomeres, fragmented and degenerating eggs.
885	1–2 cells.	Pressed between slides.	4 hrs.	10½ hrs.	20–30 cells; yolk cleavage suppressed (with multiple nuclei) or increased (with multiplication of macromeres).
886	4 cells.	Pressed between slides.	12½ hrs.	1½ hrs.	Many fragmented and degenerating; abnormal cleavages similar to preceding.
893	24 cells.	Pressed between slides.	4 hrs.	½ hr.	Few modifications.
894	1 cell.	Pressed between slides.	4 hrs.	½ hr.	1–2 cells; few modifications.
895	4 cells.	Pressed out of capsules.	4 hrs.	¾ hr.	Few eggs; few modifications.
896	30–40 cells.	Pressed in capsules.	4 hrs.	2 hrs.	Few modifications; eggs faded.
897	1 cell.	Pressed out of capsules.	4 hrs.	½ hr.	Few eggs and much faded.
898	16–24 cells.	Pressed in capsules.	4 hrs.	2 hrs.	Ca. 30 cells; few modifications.
899	16–24 cells.	Pressed in capsules.	4 hrs.	0 hrs.	24–30 cells; eggs packed together and abnormal in form.
900	1–24 cells.	Pressed out of capsules.	4 hrs.	0 hrs.	1–24 cells; macromeres may be abnormal in number, size and position.
901	1 cell.	Pressed out of capsules.	4 hrs.	0 hrs.	1–4 cells; modifications similar to preceding Fig. 48.
902	4–16 cells.	Pressed out of capsules.	4 hrs.	2 hrs.	4–20 cells; similar to No. 900.
903	1 cell.	Pressed out of capsules.	4 hrs.	2 hrs.	1–4 cells; modifications similar to No. 900.
904	1–8 cells.	Pressed in capsules.	4 hrs.	2 hrs.	1–16 cells; macromeres and micromeres may be abnormal in number, size and position. Fig. 55.
905	1 cell.	Pressed out of capsules.	4 hrs.	2 hrs.	1–8 macromeres; few eggs.
906	1–8 cells.	Pressed out of capsules.	4 hrs.	2 hrs.	1–20 cells; a few abnormal cleavage forms. Fig. 30.
907	1 cell.	Pressed out of capsules.	4 hrs.	2 hrs.	1–4 cells; unequal and non-synchronous divisions of macromeres.
908	1–24 cells.	Pressed out of capsules.	4 hrs.	4 hrs.	1–30 cells; exovates on some macromeres.
909	8–24 cells.	Pressed in capsules.	4 hrs.	4 hrs.	30–40 cells; almost all normal.

III. Effects of Pressure.—(*Continued.*)

No.	Stage.	Experiment.	Duration.	Free in Sea Water.	Results.
910	4–20 cells.	Pressed out of capsules.	4 hrs.	4 hrs.	4–24 cells; many isolated macromeres.
911	1 cell.	Pressed out of capsules.	4 hrs.	4 hrs.	1–8 macromeres, which are abnormal in size and position. Figs. 51, 52, 53.
912	4–16 cells.	Pressed in capsules.	4 hrs.	4 hrs.	24–30 cells; normal.
913	1 cell.	Pressed in capsules.	4 hrs.	4 hrs.	1–16 cells; cleavage of yolk suppressed in some eggs, increased in others (1–many macromeres), depending on axis of pressure.
914	4–16 cells.	Pressed in capsules.	4 hrs.	6 hrs.	24–30 cells; few modifications.
915	1 cell.	Pressed in capsules.	4 hrs.	6 hrs.	1–8 macromeres with corresponding micromeres; many exovates and lobes; amitosis-like division figures. Figs. 54, 56, 59, 60, 61, 65.
916	4–8 cells.	Pressed out of capsules.	4 hrs.	6 hrs.	4–24 cells; a few exovates and isolated macromeres.
917	4–16 cells.	Pressed in capsules.	4 hrs.	6 hrs.	24–30 cells; nearly normal.
918	1 cell.	Pressed out of capsules.	4 hrs.	6 hrs.	1–20 cells; many modifications in number, size and position of macromeres. Figs. 45, 46, 58.
919	1 cell.	Pressed out of capsules.	4 hrs.	6 hrs.	Similar to preceding. Fig. 77.
920	4 cells.	Pressed out of capsules.	4 hrs.	6 hrs.	8–24 cells; similar to No. 918.
1,000 (1)	1–2 cells.	Compressorium.	16 hrs.	0 hrs.	16–25 cells; more than 4 macromeres with corresponding micromeres. Fig. 62.
(2)	1–2 cells.	Compressorium.	16 hrs.	6 hrs.	16–30 cells; a few eggs with exovates and more than 4 macromeres. Fig. 63.
(3)	1–2 cells.	Compressorium.	16 hrs.	16 hrs.	52–70 cells; some isolated macromeres, showing partial cleavage. Fig. 67.
1,001 (1)	1–2 cells.	Compressorium.	15 hrs.	0 hrs.	1–24 cells; many isolated macromeres, also eggs with 1–8 macromeres; exovates. Fig. 66.
(2)	1–2 cells.	Compressorium.	15 hrs.	5½ hrs.	1–42 cells; similar to preceding.
(3)	1–2 cells.	Compressorium.	15 hrs.	15½ hrs.	52–70 cells; a few with more than 4 macromeres. Figs. 67, 76.
1,002 (1)	1–2 cells.	Pressed between slides.	5½ hrs.	0 hrs.	1–8 cells; macromeres abnormal in number, size, and position; exovate and isolated macromeres.
(2)	1–2 cells.	Pressed between slides.	5½ hrs.	7 hrs.	8–25 cells; similar to preceding. Fig. 42.
1,003	1–4 cells.	Pressed between slides.	7 hrs.	0 hrs.	2–16 cells; macromeres abnormal in number, size and position; isolated macromeres and and exovates. Figs. 57, 58.
1,004 (1)	1–2 cells.	Compressorium.	7½ hrs.	0 hrs.	1–16 cells; similar to preceding.
(2)	1–2 cells.	Compressorium.	7½ hrs.	7 hrs.	8–40 cells; similar to No. 1,002 (2).

IV. Effects of Electric Current.

1. *Porcelain-Boot Electrodes.*

No.	Stage.	Strength.	Duration.	Normal.	Results.
995 (1)	1–4 cells.	¼ volt (10 dry cells).	50 min.	0 min.	In eggs most affected spindle fibers are gone and chromosomes fused; in resting nuclei chromatin dissolved.
(2)	1–4 cells.	¼ volt.	1½ hrs.	0 min.	Many killed and fragmented; spindle fibers gone, chromosomes fused; nuclei and cytoplasm dislocated as in centrifuged eggs.
(3)	1–4 cells.	¼ volt.	1½ hrs.	0 min.	Similar to preceding.
(4)	1–4 cells.	Control on preceding.		1½ hrs.	Normal 8–16 cell stage.
(5)	1–4 cells.	¼ volt.	1½ hrs.	¾ hr.	Many killed and fragmented; spindle fibers gone and chromosomes fused in eggs most affected.
996 (1)	1–2 cells.	Beginning control.			Normal 1–2 cells; many cytasters in egg during approach of germ nuclei.
(2)	1–4 cells.	½ volt.	15 min.	2 hrs.	2–16 cells, many normal; in some, nucleus, cytoplasm, yolk displaced by convection.
(3)	1–4 cells.	½ volt.	15 min.	5 hrs.	Similar to preceding.
(4)	1–2 cells.	End control.		1½ hrs.	Normal 1–8 cells.
997 (1)	2–8 cells.	Control on 997 (2) and (3).			Normal 2–8 cells.
(2)	2–8 cells.	1 volt.	15 min.	2½ hrs.	Spindle fibers gone and chromosomes fused; nuclei, cytoplasm, yolk displaced as in centrifuged eggs. Figs. 88, 89, 91.
(3)	1–4 cells.	1 volt.	15 min.	30 min.	Similar to preceding. Figs. 80, 81, 85.
998 (1)	52 cells.	4 dry cells.	1¾ hrs.	0 min.	Micromeres spherical; cytoplasm collected on one side of nucleus.
(2)	52 cells.	4 dry cells.	1¾ hrs.	11 hrs.	Spherical micromeres, separate from macromeres—framboisia; spindle fibers gone, chromosomes fused; parts dislocated as in centrifuged eggs. Fig. 93.
(3)	Control.			12¾ hrs.	Normal 68-cell stage.
999 (1)	1–4 cells.	12 dry cells.	50 min.	1½ hrs.	Nearly normal, few eggs penetrated by current; chromatin and cytoplasm stain more faintly than in normal eggs.
(2)	1–4 cells.	12 dry cells.	30 min.	10 min.	Similar to preceding.
(3)	1–4 cells.	12 dry cells.	50 min.	40 min.	Nearly normal; eggs penetrated by current are similar to No. 997 (2).
(4)	1–4 cells.	12 dry cells.	50 min.	3 hrs.	Similar to preceding.
1,120 (1)	1–20 cells.	1 mil. amp.	45 min.	30 min.	Quite normal; current too weak.
(2)	1–20 cells.	1 mil. amp.	45 min.	24 hrs.	Ca. 52 cells; generally normal; large clear nuclei in a few eggs; sphere may be fragmented.
(3)	1–20 cells.	1 mil. amp.	45 min.	24 hrs.	Similar to preceding.
(6)	1–20 cells.	1 mil. amp.	45 min.	46 hrs.	Ca. 80 cells; quite normal.

2. *Graphite-Plate Electrodes.*

(Eggs of normal control mounted on each slide with those of experiment.)

No.	Stage.	Strength.	Duration.	Normal.	Results.
1,100	2–20 cells.	50 mil. amp.	15 min.	0 hrs.	Eggs coagulated; polarity unchanged.
1,101	2 cells.	50 mil. amp.	4 min.	17 hrs.	Ca. 25 cells; polarity unchanged; spindles normal.
1,102	12–20 cells.	40 mil. amp.	5 min.	0 hrs.	Eggs stuck together; no change in nuclei or spindle.
1,103	12–20 cells.	40 mil. amp.	5 min.	0 hrs.	Similar to preceding.
1,104	1–30 cells.	5 mil. amp.	5 min.	0 hrs.	Many eggs ruptured, blastomeres isolated, exovates due to pressure; polar rays and spindle fibers may be faint or lacking. Fig. 68.
1,105	1–8 cells.	5 mil. amp.	5 min.	0 hrs.	Similar to preceding.

IV. Effects of Electric Current.—(*Continued.*)

2. *Graphite-Plate Electrodes.*

No.	Stage.	Strength.	Duration.	Normal.	Results.
1,106	1–8 cells.	5 mil. amp.	5 min.	17 hrs.	Normal eggs 24–40 cell stage; abnormal 1–4 cell stages. Spindles gone, chromosomes fused, polarity changed; polyasters and multiple nuclei; cleavage irregular. Figs. 82–84.
1,107	1–8 cells.	5 mil. amp.	5 min.	25 hrs.	Similar to preceding; many eggs fragmented.
1,108	1–8 cells.	5 mil. amp.	5 min.	60 hrs.	Normal eggs in early gastrula; others fragmented.
1,109	1–8 cells.	5 mil. amp.	2 min.	30 hrs.	52–70 cells; many broken eggs, some polyasters and massed chromatin.
1,110 (1)	1–20 cells.	5 mil. amp.	2 min.	4 hrs.	Many normal; few show effects of pressure; others effects of convection in dislocation of cytoplasm, nuclei, spindles, and in fusion of chromosomes.
(2)	1–20 cells.	5 mil. amp.	2 min.	22 hrs.	Similar to preceding. Fig. 78.
1,111	1st maturation to 4 cells.	5 mil. amp.	1 min.	0 hrs.	Identically like control.
1,112 (1)	1–8 cells.	5 mil. amp.	1 min.	4 hrs.	Chiefly normal; a few show effects of current.
(2)	1–8 cells.	5 mil. amp.	1 min.	22 hrs.	Chiefly normal 29–52 cells; a few show effects of convection in dislocation of parts, faint spindles and fused chromosomes.
1,113 (1)	1–8 cells.	5 mil. amp.	1 min.	30 hrs.	Many normal 52–70 cells; other 1–8 cells, show effects of current.
(2)	1–8 cells.	5 mil. amp.	1 min.	60 hrs.	80–100 cells; similar to preceding.
1,114	Before maturation.	2 mil. amp.	15 min.	5 hrs.	Few eggs and deeply stained; some show effects of current in dislocation of parts.
1,115	1st maturation.	2 mil. amp.	15 min.	5 hrs.	Many killed; a few show effects of current in dissolution of chromosomes and spindles.
1,116	20–30 cells.	2 mil. amp.	10 min.	5 hrs.	Many killed; others show framboisia.
1,117	1–16 cells.	2 mil. amp.	5 min.	3 hrs.	Many eggs fragmented; a few show dislocation of parts; polyasters and irregular cleavage.
1,118	1–20 cells.	2 mil. amp.	5 min.	3½ hrs.	Some show exovates, effects of pressure; others, the effects of convection in dislocation of nuclei and cytoplasm.
1,119	2–20 cells.	2 mil. amp.	5 min.	20 hrs.	Framboisia, scattered blastomeres; many dead.
1,121 (1)	1–2 cells.	5 mil. amp.	10 min.	0 hrs.	Nearly normal; a few tetrasters.
(2)	1–2 cells.	5 mil. amp.	10 min.	3½ hrs.	Cleavage stopped in most eggs; tetrasters; spindles displaced and chromatin dissolved. Figs. 69–75, 87, 90, 92.
(3)	1–2 cells.	5 mil. amp.	10 min.	16 hrs.	Many abnormal cleavages due to pressure; multiple nuclei; eggs without chromatin have disintegrated. Fig. 79.
(4)	1–2 cells end control.			16 hrs.	Normal 24–32 cell stage.
1,122 (1)	1st maturation.	7 mil. amp.	5 min.	0 hrs.	Eggs and spindles normal; graphite plates were probably in contact.
(2)	1st maturation.*	7 mil. amp.	5 min.	1 hr.	Normal second maturation.
(3)	1st maturation.*	7 mil. amp.	5 min.	3 hrs.	Prophase first cleavage, normal.
1,123	1st cleavage.	7 mil. amp.	5 min.	0 hrs.	Normal; graphite plates were probably in contact.
1,140 (1)	1 cell.*	2 mil. amp.	2 min.	5½ hrs.	Fig. 86. Many eggs normal; others have polyasters and multiple nuclei. Some show effects of pressure, others of electric current; in latter nuclear membrane, chromatin, and spindle fibers almost dissolved; chromosomes do not dissolve as readily as resting chromatin; in a few cases nuclei and cytoplasm dislocated, probably by current.
(2)	1 cell.*	2 mil. amp.	2 min.	11 hrs.	Similar to preceding.

IV. Effects of Electric Current.—(*Continued.*)

2. *Graphite-Plate Electrodes.*

No.	Stage.	Strength.	Duration.	Normal.	Results.
1,141 (1)	1–16 cells.*	2 mil. amp.	2 min.	4½ hrs.	1–29 cells; many fragmented eggs showing partial cleavage; chromatic granules scattered throughout the yolk.
(2)	1–16 cells.*	2 mil. amp.	2 min.	10 hrs.	20–48 cells (a few older); eggs generally normal; some isolated blastomeres showing partial cleavage; many lobulated nuclei and karyomeres.
1,142 (1)	1–20 cells.*	1 mil. amp.	5 min.	4 hrs.	1–20 cells; many eggs fragmented, others fused together; cytoplasm not segregated from yolk; chromatin may be dissolved.
(2)	1–20 cells.*	1 mil. amp.	5 min.	8 hrs.	1–29 cells; many normal, others similar to preceding; yolk cleavage may be suppressed, with formation of polyasters, or increased, with formation of many macromeres.

* Water carefully drained off graphite plate; contact through eggs and egg capsules only.

V. Effects of Abnormal Temperature.

No.	Stage.	Experiment.	Duration.	Results.
960	1–4 cells.	Ca. 35° C.	4 hrs.	Eggs degenerating; chromatin of resting nuclei clumped; chromosomal vesicles scattered; cytoplasm vacuolated.
961 (1)	1–8 cells.	Ca 2°–3° C.	4 hrs.	1–8 cells, normal.
(2)	1–8 cells.	Ca 2°–3° C.	16 hrs.	4–8 cells, normal; spheres very distinct. Figs. 100–102.
(3)	1–8 cells.	Ca 2°–3° C.	28 hrs.	8–16 cells, normal; spheres very distinct with peripheral layer of coarse granules.
(4)	1–8 cells.	Ca 2°–3° C.	40 hrs.	4–30 cells; polyasters and multiple nuclei in cells where cleavage furrow was suppressed; sphere less distinct, and sphere granules scattered. Figs. 103–105.
1,170 (1)	1–2 cells.	Ca. 38° C.	1 hr.	Surface tension reduced; eggs very irregular in outline, with all archiplasm drawn into central area or in small masses under cell membrane; no astral rays or spindle fibers; chromosomes scattered. Fig. 94.
(2)	1–2 cells.	Ca. 38° C. Ca. 25° C.*	1 hr. 6 hrs.	Similar to preceding; no development.
1,171 (1)	2–24 cells.	37° C.	½ hr.	Profound changes in structure and orientation of mitotic figures; chromosomes scattered; centrosomes and spindle fibers gone; archiplasm drawn into astral areas, or collected along division walls. Figs. 96, 97.
(2)	2–24 cells.	37° C. Ca. 25° C.*	½ hr. 15 hrs.	Similar to preceding; chromosomes do not form nuclei and may be far away from cytoplasmic areas. Figs. 98, 99.
1,172 (1)	1–24 cells.	36° C.	1 hr.	Similar to 1,171 (1); archiplasm very distinct from surrounding plasma.
(2)	1–24 cells.	36° C. Ca. 23°–25°*	1 hr. 15 hrs.	Similar to 1,171 (2).
1,173 (1)	2–16 cells, a few gastrulæ.	37°	¼ hr.	Surface tension reduced; spindle fibers gone; chromosomes scattered; archiplasm not sharply segregated.
(2)	2–16 cells. Id.	37° Ca. 27°*	¼ hr. 3 hrs.	Scattered chromosomes of preceding have produced numerous karyomeres.
1,174 (1)	1 cell.	37°	¼ hr.	Surface tension reduced; mitotic figures very abnormal; spindle fibers gone; chromosomes scattered.
(2)	1 cell.	37° Ca. 27°*	¼ hr. 3 hrs.	Scattered chromosomes have produced numerous karyomeres. Fig. 95.

* Room temperature.

V. Effect of Abnormal Temperature. (*Continued.*)

No.	Stage.	Experiment.	Duration.	Results.
1,175 (1)	1–8 cells.	33°	½ hr.	Orientiaton of mitotic figure changed; spindle fibers gone; chromosomes scattered, otherwise normal in appearance.
(2)	1–8 cells.	33° Ca. 27°*	½ hr. 3 hrs.	Irregular cleavages, due to reduced surface tension; many karyomeres, due to scattered chromosomes; mitosis normal at 27°.
1,176 (1)	4–16 cells.	34°–35°	½ hr.	Sharp segregation of cytoplasm and yolk; archiplasm drawn into central areas and division walls; chromosomes scattered; centrosome and spheres indistinct.
(2)	4–16 cells.	34°–35° Ca. 23°*	½ hr. 3 hrs.	Collection of clear plasma at animal pole and along cleavage furrows; cleavage frequently suppressed; many karyomeres and irregular nuclei, each with a large nucleolus.
(3)	4–16 cells.	34°–35° Ca. 22°–25°*	½ hr. 19 hrs.	Ca. 24–30 cells; cleavages nearly normal, though sometimes suppressed; many scattered chromosomes and karyomeres.
(4)	4–16 cells.	34°–35° Ca. 22°–25°*	½ hr. 27 hrs.	Ca. 52–60 cells; similar to preceding; chromatic connections between daughter nuclei, the result of scattered chromosomes.
(5)	4–16 cells.	34°–35° Ca. 22°–25°*	½ hr. 44 hrs.	Ca. 80–90 cells; many karyomeres and irregular nuclei which persist through many mitoses but do not interfere with regular cleavages.
1,177 (1)	1–2 cells.	33°–34°	½ hr.	Daughter nuclei and clear plasma drawn down into middle of egg along cleavage furrow; latter often suppressed at vegetal pole and not at animal; some scattered chromosomes.
(2)	1–2 cells.	33°–34° Ca. 22°–25°*	½ hr. 17 hrs.	Ca. 30–40 cells; second cleavage may be suppressed, and polyasters may be present; many karyomeres, especially in entomeres.

* Room Temperature.

VI. Effects of Ether Added to Sea Water.

No.	Stage.	Strength, Per Cent.	Duration.	Results.
800	1–8 cells.	1	1 hr.	No modifications, except that cytoplasm and yolk are not sharply separated.
801	1–8 cells.	½	5½ hrs.	4–16 cells; similar to preceding. Control in 2–8 cells.
803	1–8 cells.	1	5½ hrs.	4–16 cells; similar to No. 800.
818	2–20 cells.	1	25 hrs.	Ca. 52 cells; archiplasm gathered around sphere or nucleus; chromatin clumped; chromosomes scattered and spindle fibers gone; no signs of amitosis.
819	2–20 cells.	1	25 hrs.	Similar to preceding; control in 52 cell stage.
877	1–8 cells.	3	¼ hr.	Cleavage stopped; yolk and cytoplasm intermingle; archiplasm gathers along cleavage furrows; chromosomes scattered; spindle fibers indistinct.
878	1–8 cells.	3 (Normal S. W. 6 hrs.)	3 hrs.	Similar to preceding; sphere granules widely scattered; centrosomes large; chromatin clumped in resting nuclei; archiplasm gathers in cleavage furrows; blastomeres may separate.
1,180 (1)	1–2 cells.	2	2 hrs.	Many lobes and deep furrows in resting stages which bear no constant relations to egg axes or nuclei; clear plasma bounds furrows; mitotic figures approximately normal, spindle fibers distinct.
(2)	1–2 cells.	2	6 hrs.	4–16 cells; many eggs normal; a few show large polar bodies and many macromeres; mitotic figures normal. All ether had evaporated before close of experiment.
1,181 (1)	1–20 cells.	1	1 hr.	Cleavage and mitotic figures entirely normal; eggs stain diffusely.
(2)		1	16 hrs.	16–52 cells; cleavage and mitoses approximately normal; eggs are swollen and stain diffusely; deeply staining granules between yolk spheres.

VII. Effects of Reduced Oxygen Tension.

1. *Sea Water Boiled and Cooled.*

a. In Open Bottles.

No.	Stage.	Duration.	Normal Sea Water.	Results.
935	1–2 cells.	12 hrs.	0 hrs.	16–30 cells, normal.
936	16–24 cells.	12 hrs.	0 hrs.	Ca. 50 cells; normal.
937	24–30 cells.	12 hrs.	0 hrs.	Ca. 64 cells, normal.
938	1 cell.	18 hrs.	0 hrs.	25–60 cells, normal.
939	4–12 cells.	18 hrs.	0 hrs.	52 cells, normal.
940	24–30 cells.	18 hrs.	0 hrs.	Ca. 64 cells, normal.
947	1 cell.	48 hrs.	0 hrs.	1–30 cells; framboisia; fusing eggs; many dead.
948	4–12 cells.	48 hrs.	0 hrs.	52–70 cells; framboisia; isolated macromeres; many dead.
949	24–30 cells.	48 hrs.	0 hrs.	64–86 cells; similar to preceding.
950	1–16 cells.	4 hrs.	0 hrs.	2–20 cells, normal.
951	1–16 cells.	17 hrs.	0 hrs.	48–60 cells, nearly normal; some chromosomes scattered, and some resting nuclei lobulated or multiple.
952	1–16 cells.	24 hrs.	0 hrs.	52–70 cells, nearly normal.
953	1–16 cells.	40 hrs.	0 hrs.	64–86 cells, nearly normal; sometimes only one blastomere of 2-cell stage develops.

b. In Stoppered Bottles.

1,010	1–4 cells.	36 hrs.	0 hrs.	1–4 cells; cleavage stopped; eggs smaller than normal, only 120 μ in diameter; nuclei large, vesicular, with large nucleoli; centrosomes very large; sphere granules widely scattered. Figs. 106–109.
1,011	1 cell.	5 hrs.	0 hrs.	1–4 cells; astral rays withdrawn and spheres very distinct; otherwise typical.
1,012	1–4 cells.	6 hrs.	0 hrs.	1–20 cells, nearly normal.
1,013	2–20 cells.	20 hrs.	0 hrs.	2–20 cells; nuclei large, with chromatin clumped.
1,014	Gastrulæ.	20 hrs.	0 hrs.	No changes.
1,015	1–8 cells.	24 hrs.	0 hrs.	1–30 cells (a few older stages accidentally included); big nuclei with chromatin clumped; spindles faint.
1,016	1–8 cells.	27 hrs.	0 hrs.	1–20 cells; nuclei large and achromatic; nucleoli large; sphere granules scattered. Figs. 112, 114.
1,017	1–8 cells.	48 hrs.	0 hrs.	1–20 cells; big nuclei and nucleoli; chromatin clumped; sphere granules scattered. Figs. 113, 117.
1,018	1 cell to gastrula.	48 hrs.	0 hrs.	1 cell to gastrula; similar to preceding; cleavage stopped in early stages, later stages not so much affected.
1,020	1–20 cells.	20 hrs.	3 hrs.	2–24 cells; normal; no development while in boiled sea water.
1,021	1–20 cells.	24 hrs.	8 hrs.	8–40 cells, normal; no development while in boiled sea water.
1,022	2–20 cells.	20 hrs.	9 hrs.	24–52 cells; similar to preceding.

2. *Partial Atmosphere of Hydrogen.*

No.	Stage.	Hydrogen Stream.	Closed Bottle.	Open Bottle.	Results.
879	1–40 cells.	3 hrs.			1–40 cells; nuclei of older stages normal; of earlier stages, large, achromatic, with large nucleoli.
880	1–30 cells.	4½ hrs.			1–30 cells; same as preceding.
881	1–30 cells.	Control.		4½ hrs.	4–48 cells, normal.
882	1–30 cells.	7½ hrs.			1–30 cells; same as No. 879.
883	1–30 cells.	Control.		7½ hrs.	24–52 cells, normal.
1,023	2–20 cells.	2 hrs.		2 hrs.	2–24 cells; nuclei and nucleoli large, chromatin clumped; few polyasters; centrosomes and spindles distinct. Fig. 115.
1,024	2–20 cells.*	2 hrs. 1 hr.	18 hrs.	3 hrs.	2–24 cells; similar to preceding; centrosomes and spindles unusually distinct.
1,025	2–20 cells.*	1 hr. 1 hr.	18 hrs.	3 hrs.	2–24 cells; similar to preceding. Figs. 110, 111, 117, 118.
1,026	2–20 cells.*	1 hr. 1 hr.	20 hrs.	5 hrs.	No development while in hydrogen; after 20 hrs. in fresh sea water eggs had reached 48–86 cells.
1,027	1–20 cells.	H 60%—Air 40%	12 hrs.		1–20 cells; cleavage stopped; nuclei nearly normal.
1,028	2–4 cells.	H 70%—Air 30%	12 hrs.		2–4 cells; cleavage stopped.
1,029	1–20 cells.	H 70%—Air 30%	24 hrs.		1–20 cells; similar to preceding.

* Eggs from two experiments mounted together.

3. *Stoppered Bottles of Normal Sea Water.*

No.	Stage.	Duration.	Results.
941	2–8 cells.	20 days.	4 cells to veliger; in early stages, division may be permanently halted in metaphase and anaphase; framboisia; exogastrulæ.
946	1–8 cells.	20 days.	Same as preceding.
1,019	1–20 cells.	48 hrs.	1–30 cells; cleavage checked; nuclei large and clear, with chromatin clumped, as in boiled sea water.

VIII. Effects of Carbonic Acid.

No.	Stage.	Sea Water.		Duration.	Results.
		Charged.	Normal.		
1,163	1–8 cells.	1 part	1 part	28 hrs.	Cell membranes usually granular and wrinkled; lobes and spinning threads present; surface tension reduced; nuclear membrane also wrinkled and lobulated, giving appearance of amitosis; chromatin dense and achromatin scanty in resting nuclei; resting spheres lie at surface of cell, as in normal eggs; mitotic figures normal; cleavage very irregular and often suppressed. Figs. 119, 120, 122–129, 131.
1,164	1–8 cells.	1	1	28 hrs.	Same as preceding.
1,165	1–8 cells.	1	1	28 hrs.	Same as preceding.
1,166	1–30 cells.	1	0	28 hrs.	Similar to preceding; resting nuclei much lobulated, as if in amitosis.
1,167 (1)	1 cell.	1	1	7 hrs.	1–8 cells; cell membrane wrinkled; yolk cleavage often suppressed; nuclei of macromeres densely chromatic; mitotic figures normal; positions of spheres, nuclei and cytoplasm dependent upon direction of cell constriction.
(2)	1 cell.	1	1	28 hrs.	20–40 cells; similar to preceding; many abnormal cleavages due to local reductions of surface tension.
1,168 (1)	1–30 cells.	1	1	4½ hrs.	1–30 cells; in addition to preceding modifications chromosomes may be widely scattered and cytoplasm coarsely granular.
(2)	1–30 cells.	1	1	20 hrs.	4–52 cells; similar to preceding; many karyomeres and irregular nuclei.
1,169 (1)	1–2 cells.	1	1	18 hrs.	1–24 cells; cleavage may be normal or very abnormal; some micromeres and nuclei go through center of egg to vegetal pole; many blastomeres lobulated; cleavage sometimes suppressed; eggs often stick together.
(2)	1–2 cells.	1	1	25 hrs.	24–48 cells; macromeres may be separated from one another and micromeres may go through center of egg to vegetal pole.
(3)	1–2 cells.	1	1	42 hrs.	64–68 cells; many eggs fused in pairs; fusion always between entomeres; nuclei always at surface and not buried along line of fusion.
1,169a(1)	1–4 cells.	1	1	3 hrs.	1–4 cells; chromatin clumped in resting nuclei; sphere outlines very distinct, especially on side away from animal pole; chromosomes scattered; in first cleavage yolk lobe and polar bodies are carried in with furrow to *Zwischenkörper*, and no new membrane is formed until after constriction is completed.
(2)	1–4 cells.	1	1	26 hrs.	8–25 cells; cleavage normal in most eggs; suppressed in a few.
(3)	1–4 cells.	1	1	44 hrs.	48–52 cells; normal in most cases; first and second cleavages suppressed in a few eggs.
(4)	1–4 cells.	1	1	50 hrs.	52–64 cells; similar to preceding.
1,169b(1)	1–4 cells.	1	1	6 hrs.	1–8 cells; some cleavages suppressed; many spindles displaced and chromosomes scattered; many spindles distorted; many lobes on cells.
(2)	1–4 cells.	1	1	24 hrs.	1–8 cells; similar to preceding; cells and nuclei lobulated; many scattered chromosomes and karyomeres, suppressed cleavages and distorted spindles.
(3)	1–4 cells.	1	1	24 hrs.	
		Normal sea water		11 hrs.	8–30 cells; many irregular cleavages with lobulated and separated macromeres.
(4)	1–4 cells.	1	1	24 hrs.	
		Normal sea water		25 hrs.	30–50 cells; cleavage very abnormal; many eggs fused.
(5)	1–4 cells.	1	1	24 hrs.	
		Normal sea water		48 hrs.	60–80 cells; very abnormal cleavage; many fused eggs.

IX. Effects of Diluting Sea Water with Fresh Water.

No.	Stage.	Dilution.		Time in		Results.
		Salt.	Fresh.	Dilute.	Normal.	
858	2–4 cells.	1 part	1 part	2 hrs.	14 hrs.	24–30 cells; yolk cleavage sometimes suppressed, polyasters, multiple nuclei; chromosomes may be scattered along spindle and form chromatic connection between daughter nuclei; older stages normal, or nearly so. Figs. 145, 147, 152, 153, 154, 157, 158.
859	2–4 cells.	1	2	1 hr.	4 hrs.	Many eggs bursted and chromatin of nuclei dissolved; no separation between yolk and cytoplasm; chromatic connection between daughter nuclei; nucleoli large. Fig. 141.
870	1 cell.	1	1	½ hr.	1½ hrs.	1–2 cells; few abnormalities.
871	1–4 cells.	1	1	1 hr.	12 hrs.	1–30 cells; many isolated macromeres showing partial cleavage; blastomeres may be abnormal in size, position and quality; yolk cleavage often suppressed; polyasters and multiple nuclei. Fig. 156.
872	1 cell.	1	1	½ hr.	15 hrs.	1–25 cells; cleavage may be adequal and little difference between macromeres and micromeres; yolk cleavage increased; direction of cleavage may be modified; spindles may be degenerate and connective fibers chromatic. Figs. 136, 142, 143.
873	1 cell.	1	1	½ hr.	24 hrs.	Similar to preceding; many eggs fragmented and degenerate.
874	4–20 cells.	1	1	1 hr.	25 hrs.	Ca. 52 cells; a few partial eggs, others normal.
875	1–8 cells.	1	2	½ hr.	7 hrs.	1–24 cells; isolated macromeres; in some cases yolk cleavage suppressed, in others increased; 1–many macromeres; polyasters, degenerate spindles, multiple spheres; tendency to equal cleavage of yolk; enormous polar body. Figs. 39, 132–135, 137–140, 144.
876	4–8 cells.	1	2	½ hr.	20 hrs.	24–32 cells, many normal, others fragmented; chromatic connection between daughter nuclei.
954	2–20 cells.	1	1	3 hrs.	0 hrs.	Few eggs, showing few abnormalities.
955	2–20 cells.	1	1	3 hrs.	14 hrs.	Similar to preceding.
956	2–20 cells.	1	1	2 hrs.	36 hrs.	Ca. 68 cells; many show polyasters, abnormal distribution of chromosomes, multiple nuclei. Fig. 150.
993 (1)	1–4 cells.	1	1	1½ hrs.	15½ hrs.	24–42 cells; in some eggs yolk cleavage suppressed with formation of polyasters, karyomeres, and multiple nuclei; in others yolk cleavage increased. Figs. 146, 148, 149, 151, 155.
(2)	1–4 cells.	1	1	1¾ hrs.	0 hrs.	1–4 cells; some isolated blastomeres; chromosomes scattered; position and direction of spindles changed.
(3)	1–4 cells.	1	1	1¾ hrs.	27 hrs.	Ca. 52 cells; irregular cleavage; yolk cleavage may be suppressed, giving rise to polyasters, multiple nuclei and discoidal cleavage, or it may be increased, macromeres and micromeres becoming equal in size.
1,182 (1)	1–4 cells.	1	1	2 hrs.	0 hrs.	1–4 cells; no sharp distinction between yolk and cytoplasm; many yolk spheres have escaped through cell membrane; lobes and deep furrows at animal pole; large polar bodies; nuclei large, centrosomes and spheres small and dense; scattered chromosomes and karyomeres; cleavages may be suppressed and nuclei displaced and macromeres separated.
(2)	1–4 cells.	1	1	2 hrs.	18 hrs.	16–52 cells; many normal, others very abnormal, with equatorial and meroblastic cleavages, and with irregular nuclei and karyomeres.
(3)	1–4 cells.	1	1	2 hrs.	28 hrs.	52–70 cells; many normal, others similar to preceding.

IX. Effects of Diluting Sea Water with Fresh Water.—(*Continued.*)

No.	Stage.	Dilution.		Time in		Results.
		Salt.	Fresh.	Dilute.	Normal.	
(4)	1–4 cells.	1 part	1 part	2 hrs.	44 hrs.	100–120 cells; similar to preceding.
(5)	1–4 cells.	1	1	2 hrs.	52 hrs.	Early gastrulæ; many normal; some framboisia; karyomeres in endoderm cells.
1,183 (1)	24–48 cells.	1	1	7½ hrs.	0 hrs.	Large cleavage cavity between macromeres and micromeres; scattered chromosomes, irregular nuclei, daughter nuclei do not become achromatic.
(2)	24–48 cells.	1	1	7½ hrs.	16½ hrs.	40–70 cells; cleavage cavity enormous, micromeres arched over it and nearly separated from yolk; karyomeres, irregular nuclei; dividing cells may contain heaps of peripheral granules.
1,184 (1)	2–52 cells.	1	1	3 hrs.	3½ hrs.	Large cleavage cavity; a few eggs show scattered chromosomes, karyomeres, and lobulated nuclei.
(2)	2–52 cells.	1	1	3 hrs.	23 hrs.	Ca. 52–80 cells; similar to preceding; exogastrulæ.
(3)	2–52 cells.	1	1	3 hrs.	28 hrs.	Ca. 52–80 cells; similar to preceding.
(4)	2–52 cells.	1	1	3 hrs.	52 hrs.	Early gastrula stage; many exogastrulæ.
1,185 (1)	1 cell.	40	60	3 hrs.	0 hrs.	1 cell; many eggs bursted; others are swollen and show little distinction between cytoplasm and yolk; nuclei swollen and achromatic; no mitotic figures.
(2)	1 cell.	40	60	3 hrs.	15 hrs.	1–20 cells; cleavage generally normal; in a few cases macromeres isolated, or cleavage suppressed.
(3)	1 cell.	40	60	3 hrs.	40 hrs.	Ca. 40–50 cells, normal.
1,186 (1)	1–8 cells.	1	1	3 hrs.	15 hrs.	Ca. 8–30 cells; in many eggs yolk cleavage suppressed; some blastomeres abnormal in position or size, others isolated; scattered chromosomes, chromatic connections between daughter nuclei, karyomeres, synkaryonts.
(2)	1–8 cells.	1	1	3 hrs.	39 hrs.	Ca. 40–60 cells; cleavage often abnormal or suppressed; many fused and partial eggs; scattered chromosomes, karyomeres and synkaryonts are abundant.
(3)	1–8 cells.	1	1	3 hrs.	50 hrs.	Ca. 50–70 cells; similar to preceding.
(4)	1–8 cells.	1	1	3 hrs.	64 hrs.	Ca. 70 cells to early gastrula; many partial or fused eggs; scattered chromosomes, karyomeres and synkaryonts.
(5)	1–8 cells.	1	1	3 hrs.	87 hrs.	Early and late gastrulæ; some normal, others abnormal; some eggs fused, others partial; karyomeres and synkaryonts.

X. Effects of Hypertonic Sea Water.

1. *NaCl Added to Sea Water.*

No.	Stage.	Per Cent.	Time in		Results.
			Solution.	Normal S. W.	
804	1–4 cells.	1	4 hrs.	0 hrs.	A spindle forms in connection with each germ nucleus, if the poles lie close together triasters or tetrasters form; no vesicular nuclei. Figs. 159–170, 210.
805	1–4 cells.	2	4 hrs.	0 hrs.	Chromatin clumped in center of nuclear vesicle; achromatin fills nuclear vesicle and in mitosis fills amphiaster; spindles shrunken and no fibers visible; chromosomes scattered; cytasters, but no multipolar spindles. Figs. 183, 189, 190.
806	1–4 cells.	3	4 hrs.	0 hrs.	Chromatin clumped in nuclear vesicle; nuclei and spindles shrunken; cytasters, but no multipolar spindles; connective fibers form spindle, widest in middle; achromatin as in preceding, divides amitotically; no plasma division.
807	1–4 cells.	4	4 hrs.	0 hrs.	Chromatin not clumped, but forms a heavy reticulum, or spireme; nuclei a little shrunken; cleavage stopped.
808	1–2 cells.	1	15½ hrs.	0 hrs.	2–20 cells; yolk cleavage largely suppressed; nuclei densely chromatic, not vesicular; polyasters, karyomeres. Figs. 195, 196.
809	1–2 cells.	2	15½ hrs.	0 hrs.	Chromatin in dense masses; achromatin in numerous separate vesicles, widely scattered, formed from substance of spindles and rays; no spindles; no cleavage. Figs. 184–188.
810	1–4 cells.	3	15½ hrs.	0 hrs.	Chromosomes do not form vesicles, but achromatin of spindle and asters does as in Figs. 79–82; no cleavage. Figs. 178–182.
811	1–2 cells.	4	15½ hrs.	0 hrs.	No cleavage; chromatin and achromatin separate and distinct; chromosomes scattered; spindle of homogeneous achromatin, no fibers; no radiations.
813	1–20 cells.	1	1 hr.	17 hrs.	8–20 cells; blastomeres abnormal in size and position; in resting stage chromatin like spireme; sphere has very definite outline, centrosomes dark and dense in sphere; mitoses few but distinct; chromosomes do not form vesicles, but achromatin and membrane form around them.
814	1–20 cells.	2	1 hr.	17 hrs.	8–25 cells, mostly normal; abnormal only when mitosis has been disturbed; no polyasters, some karyomeres; scattered chromosomes; chromatic connections between daughter nuclei in few cases. Fig. 221.
815	1–20 cells.	3	1 hr.	17 hrs.	8–25 cells; cleavage normal, development stopped only temporarily; mitoses normal; no polyasters.
816	1–20 cells.	4	1 hr.	17 hrs.	Similar to preceding.
821	1–2 cells.	1	16 hrs.	8 hrs.	25–40 cells; cleavage very irregular; yolk cleavage may be suppressed or increased; polyasters and karyomeres.
822	1–2 cells.	2	16 hrs.	8 hrs.	1–8 cells; little cleavage and very irregular; polyasters and karyomeres. Figs. 174, 175, 177, 217.
823	1–2 cells.	3	16 hrs.	8 hrs.	1–2 cells; no cleavage, no asters, no centrosomes or spheres; nuclei may be enormous and chromatic, or small and clear; the latter, which were in mitosis at beginning of exp., have not taken in achromatin, which is scattered as homogeneously colored spherules. Figs. 171–173, 176, 191, 192.
824	1–2 cells.	4	16 hrs.	8 hrs.	1–2 cells; similar to preceding; resting nuclei constricted and lobulated as in amitosis; big nucleoli; astral rays contracted, spindle fibers fused, chromosomes scattered; achromatic spherules widely scattered.

X. Effects of Hypertonic Sea Water.—(*Continued.*)

1. *NaCl Added to Sea Water.*

No.	Stage.	Per Cent.	Time in		Results.
			Solution.	Normal S. W.	
825	1–2 cells.	2	16 hrs.	23 hrs.	1–2 cells; no cleavage; karyomeres, polyasters; some nuclei large, thin walled and achromatic, others contain large, dense nucleolus; many nuclei constricted and lobed as in amitosis. Fig. 202.
827	1–20 cells.	¾	9 hrs.	8 hrs.	Ca. 2–50 cells; cleavage irregular; yolk cleavage usually suppressed, sometimes increased; karyomeres; many polyasters; later stages less modified than earlier ones. Figs. 203, 206.
828	1–20 cells.	1	2 hrs.	6½ hrs.	1–24 cells; very abnormal cleavage; yolk cleavage suppressed, plasma cleavage active; polyasters, scattered chromosomes, karyomeres. Fig. 222.
829	1–20 cells.	1½	9 hrs.	18 hrs.	Ca. 1–60 cells; cleavage very irregular; yolk divides seldom; many polyasters, karyomeres.
830	1–20 cells.	2	9 hrs.	32 hrs.	Ca. 1–70 cells; cleavage nearly suppressed; many polyasters, karyomeres, which stain faintly; clear, unstained nucleoli. Fig. 207.
831	1–20 cells.	3	2 hrs.	6½ hrs.	Ca. 1–30 cells; no polyasters or karyomeres, chromosomes widely scattered; nuclei flattened in polar axis.
832	1–20 cells.	4	2 hrs.	6½ hrs.	1–20 cells; yolk cleavage suppressed; karyomeres, polyasters; nuclei flattened in polar axis; advanced stages nearly normal.
839	1–4 cells.	1	6 hrs.	20 hrs.	Ca. 1–30 cells; yolk cleavage suppressed; polyasters, karyomeres; scattered chromosomes; bunch of micromeres on unsegmented yolk.
840	1–4 cells.	2	6 hrs.	20 hrs.	Ca. 1–30 cells; yolk cleavage suppressed; polyasters, karyomeres; chromosomes widely scattered.
843	1–4 cells.	1	6 hrs.	15 hrs.	30–40 cells; yolk cleavage suppressed; polyasters, karyomeres; more advanced stages normal. Fig. 205.
844	1–4 cells.	2	6 hrs.	15 hrs.	Ca. 2–40 cells; similar to preceding.
861	1–4 cells.	½	13 hrs.	0 hrs.	20–40 cells; almost normal; yolk cleavage occurs; few polyasters.
862	1 cell.	1	4 hrs.	10 hrs.	Ca. 2–24 cells; yolk cleavage more or less suppressed; many polyasters and karyomeres; heaps of micromeres.
863	1–4 cells.	2	2½ hrs.	10 hrs.	1–25 cells; advanced stages normal; yolk cleavage suppressed; many polyasters and heaps of micromeres. Figs. 204, 213.
864	1–4 cells.	3	2 hrs. 3½ hrs.	10 hrs. 10 hrs.	Ca. 1–30 cells; cleavage irregular; yolk cleavage more or less suppressed; many polyasters and karyomeres Fig. 211.
865	1–4 cells.	4	2 hrs.	10 hrs.	Ca. 1–30 cells; cleavage less irregular than preceding; few polyasters and karyomeres; later stages normal. Figs. 209, 212.
965	20–30 cells.	1	3 hrs.	0 hrs.	20–30 cells; plasma, nuclei and spindles shrunken and plasmolyzed.
966	20–30 cells.	1	16 hrs.	0 hrs.	20–36 cells; little if any cleavage; plasma, nuclei, and spindles much shrunken and plasmolyzed.
967	1–20 cells.	1	16 hrs.	12 hrs.	Ca. 1–40 cells; earlier stages show suppressed, or increased, yolk cleavage; polyasters, and karyomeres; scattered chromosomes, chromatic connections between daughter nuclei; older stages less abnormal.
968	1–20 cells.	1	16 hrs.	24 hrs.	1–64 cells; abnormalities similar to preceding; some eggs fused together, others with isolated macromeres.
969	4–12 cells.	2	3 hrs.	0 hrs.	4–12 cells; plasma, nuclei and spindles shrunken; in resting nuclei chromatin clumped in middle of vesicle of achromatin; in mitosis amphiaster lies in definite area of achromatin; plasma gathers in cleavage furrows.

X. Effects of Hypertonic Sea Water.—(*Continued.*)

1. *NaCl Added to Sea Water.*

No.	Stage.	Per Cent.	Time in Solution.	Time in Normal S. W.	Results.
970	4–12 cells.	2	16 hrs.	0 hrs.	4–12 cells; similar to preceding, but modifications more pronounced.
971	4–12 cells.	2	16 hrs.	12 hrs.	4–12 cells; great numbers of karyomeres and constricted nuclei, polyasters.
972	4–12 cells.	2	16 hrs.	24 hrs.	4–20 cells; very abnormal cleavage; yolk cleavage sometimes suppressed, sometimes increased; many polyasters and karyomeres. Figs. 220, 223.
973	2–20 cells.	3	1 hr.	1 hr.	2–20 cells; nearly normal; few cells show any signs of plasmolysis.
974	2–20 cells.	3	1 hr.	16 hrs.	24–44 cells; mostly normal; in a few eggs yolk cleavage suppressed, polyasters and karyomeres.
975	2–20 cells.	3	1 hr.	24 hrs.	30–52 cells; similar to preceding.
976	2–20 cells.	3	1 hr.	39 hrs.	60–70 cells; almost all normal; a few karyomeres.
977	2–20 cells.	4	1 hr.	1 hr.	2–20 cells; plasma, nuclei, spindles are normal; have recovered from plasmolysis.
978	2–20 cells.	4	1 hr.	16 hrs.	20–50 cells; plasma, nuclei and spindle normal.
979	2–20 cells.	4	1 hr.	24 hrs.	20–60 cells; chiefly normal; a few karyomeres.
980	2–20 cells.	4	1 hr.	39 hrs.	60–70 cells; chiefly normal; a few degenerating mitotic figures which never recovered; few karyomeres.
981	1–20 cells.	5	¾ hr.	2 hrs.	1–20 cells; chiefly normal; few signs of plasmolysis; a few chromatic connections between daughter nuclei.
982	1–20 cells.	5	½ hr. – ¾ hr.	18 hrs.	24–44 cells; chiefly normal; no polyasters or karyomeres.
983	1–20 cells.	5	¾ hr.	24 hrs.	2–52 cells; mainly normal; some earlier stages abnormal, with suppressed or increased yolk cleavage, polyasters and karyomeres.
984	1–20 cells.	5	¾ hr.	40 hrs.	60–70 cells, mainly normal; a few abnormalities of earlier stages similar to preceding.
985	2–20 cells.	6	¾ hr.	2 hrs.	8–24 cells, mainly normal; plasma, nuclei, spheres, spindles show no trace of plasmolysis.
986	2–20 cells.	6	½ hr. – ¾ hr.	18 hrs.	2–40 cells, mainly normal; some early stages show suppression of yolk cleavage, with polyasters and karyomeres; later stages are normal.
987	1 cell.	6	¾ hr.	24 hrs.	1–4 cells; suppressed cleavage, polyasters, karyomeres.
988 (1)	1–4 cells.	1	2 hrs.	0 hrs.	1–4 cells; no cleavage; nuclei and spindles shrunken; achromatin withdrawn from radiations into spindle, leaving isolated portions of achromatin, which become cytasters.
(2)	1–4 cells.	1	4 hrs.	0 hrs.	1–4 cells; similar to preceding.
(3)	1–4 cells.	1	5 hrs.	0 hrs.	1–4 cells; similar to preceding.
989 (1)	1 cell. 2d Maturation	1	4¾ hrs.	0 hrs.	1 cell; nuclei and spindles shrunken; cytasters.
(2)	1–4 cells.	1	4¾ hrs.	0 hrs.	1–4 cells; plasma, nuclei and spindles shrunken; few, if any, cytasters.
(3)	1–4 cells.	1	4¾ hrs.	13 hrs.	1–16 cells; suppressed and unequal cleavage of yolk; polyasters, karyomeres; enormous asters surrounded by many chromosomes.
990 (1)	1–4 cells.	4	2¾ hrs.	13½ hrs.	2–16 cells; scattered chromosomes; chromatic connections between daughter nuclei; polyasters, karyomeres, big nuclei and nucleoli.
(2)	1–4 cells.	1	2¾ hrs.	13½ hrs.	24–44 cells; increased cleavage of yolk.
(3)	1–4 cells.	4	2¾ hrs.	24 hrs.	1–24 cells; yolk cleavage may be suppressed in 1st and 2d cleavages, increased in later cleavages; polyasters, karyomeres; no normal mitoses.
(4)	1–4 cells.	1	2¾ hrs.	24 hrs.	52–60 cells; mostly normal.
(5)	1–4 cells.	1	2¾ hrs.	36 hrs.	68–76 cells; a few karyomeres and polyasters.

X. Effects of Hypertonic Sea Water.—(*Continued.*)

1. *NaCl Added to Sea Water.*

No.	Stage.	Per Cent.	Time in		Results.
			Solution.	Normal S. W.	
(6)	1–4 cells.	4	2¾ hrs.	36 hrs.	4–60 cells; first and second cleavage of yolk may be suppressed; karyomeres, polyasters.
994 (1)	1–20 cells.	1	2¾ hrs.	8 days.	Ca. 100–150 cells; nearly normal; few karyomeres.
(2)	1–20 cells.	1	4 hrs.	0 hrs.	1–20 cells; no cleavage; cytasters; polyasters.
(3)	1–20 cells.	1	4 hrs.	0 hrs.	1–20 cells; plasma, nuclei and spindles shrunken; no cleavage; many cytasters and polyasters.
(4)	1–20 cells.	1	4¾ hrs.	36 hrs.	1–40 cells; very irregular cleavage; karyomeres, polyasters.
(5)	1–2 cells.	1	5 hrs.	0 hrs.	1–2 cells; plasma, nuclei and spindles shrunken; scattered chromosomes; polyasters.
1,187 (1)	1–2 cells.	1	4 hrs.	0 hrs.	1–4 cells; cytasters are present during approach of pronuclei; some furrows suppressed.
(2)	1–2 cells.	1	4 hrs.	19 hrs.	Ca. 30–40 cells; yolk cleavage may be suppressed; macromeres may be isolated; karyomeres.
(3)	1–2 cells.	1	4 hrs.	30 hrs.	Ca. 60–70 cells; similar to preceding.
(4)	1–2 cells.	1	4 hrs.	44 hrs.	Ca. 80–100 cells; karyomeres or lobulated nuclei always occur where yolk cleavage is suppressed.
(5)	1–2 cells.	1	4 hrs.	67 hrs.	Gastrula stages; similar to preceding.

2. *$MgCl_2$ Added to Sea Water.*

No.	Stage.	Per Cent.	Time in		Results.
			Solution.	Normal S. W.	
833	1–2 cells.	2	9 hrs.	9 hrs.	First and second cleavages of yolk suppressed; heap of micromeres on unsegmented yolk; polyasters, karyomeres; very abnormal cleavage. Figs. 200, 208.
834	1–2 cells.	3	9 hrs.	9 hrs.	Similar to preceding. Fig. 199.
835	1–2 cells.	4	9 hrs.	9 hrs.	Similar to preceding; many micromeres large and contain yolk; spindles deep in yolk.
841	1–2 cells.	2	5 hrs.	17 hrs.	Yolk cleavage partially suppressed; heap of micromeres on unsegmented yolk; polyasters, karyomeres; chromosomes scattered.
842	1–4 cells.	4	5 hrs.	17 hrs.	First and second cleavages of yolk largely suppressed; 26–36 cells in eggs which had passed 4-cell stage; heap of micromeres; polyasters, karyomeres. Figs. 197, 218.
845	1–4 cells.	2	4 hrs.	16 hrs.	24–36 cells in eggs which had passed 4-cell stage, nearly normal; if first and second cleavages suppressed, heap of micromeres on yolk.
846	1–4 cells.	4	4 hrs.	16 hrs.	First and second yolk cleavages suppressed; heap of micromeres; polyasters, karyomeres; 24–36 cells of normal types. Figs. 214–216.
866	1 cell.	1	14½ hrs.	0 hrs.	24 cells; normal except that plasma and chromatin are shrunken a little.
867	1–8 cells.	8	¾ hr.	6½ hrs.	First and second yolk cleavages suppressed; polyasters, karyomeres; 16–24 cells in normal types. Figs. 41, 219.
868	1–4 cells.	8	¾ hr.	10½ hrs.	First and second cleavages suppressed; heap of micromeres; polyasters, karyomeres; many nuclei lobed, constricted, double; older stages normal.
991	1–8 cells.	1	18 hrs.	0 hrs.	First and second cleavages suppressed; spindles and nuclei shrunken; ca. 24 cells of normal type. Figs. 198.
992 (1)	1–8 cells.	4	3 hrs.	1 hr.	First and second cleavages suppressed; karyomeres due to failure of chromosomes to fuse.
(2)	1–8 cells.	4	3 hrs.	14½ hrs.	1–2-cell stages have produced very abnormal forms; 2–8-cell stages have produced normal forms with 29–44 cells.
(3)	1–8 cells.	4	3 hrs.	27 hrs.	Similar to preceding; late stages with 64–70 cells; karyomeres.

X. Effects of Hypertonic Sea Water.—(*Continued.*)

3. *KCl Added to Sea Water.*

No.	Stage.	Per Cent.	Time in Solution.	Time in Normal S. W.	Results.
836	1–4 cells.	¾	9 hrs.	35 hrs.	First and second yolk cleavages may be suppressed, with formation of polyasters and karyomeres; after 4-cell stage fewer micromeres than normal may be formed, with nuclei far apart.
837	1–4 cells.		9 hrs.	35 hrs.	1–4 cells; cleavage largely suppressed, constricted and multiple nuclei, with big nucleoli. Figs. 173, 193, 194.
838	1 cell.		9 hrs.	18 hrs.	All cleavage suppressed; lobed, constricted, and multiple nuclei; little distinction between plasma and yolk.
847	1–4 cells.	1½	5 hrs.	16 hrs.	25–42 cells; a few still in 1–4-cell stage; nuclei may be multiple in macromeres and single in micromeres; no sharp distinction between yolk and plasma.
847*a*	1–4 cells.	1½ 1	5 hrs.	16 hrs.	2–24 cells; yolk cleavage and mitosis in macromeres very abnormal; scattered chromosomes, polyasters; karyomeres, especially in macromeres.

4. *Herbst's Ca-free Sea Water.** 30.0 g. NaCl
.8 KCl
6.6 $MgSO_4$
962.2 dist. water.

No.	Stage.	Per Cent.	Time in Solution.	Time in Normal S. W.	Results.
848	1–20 cells.		18 hrs.	0 hrs.	1–30 cells; plasma, nuclei and spindles somewhat shrunken; cytasters in unsegmented egg, always connected with radiations; chromatin is like a coarse spireme; chromosomes fail to fuse in telophase; karyomeres; polyasters. Fig. 201.

5. *Cane Sugar Added to Sea Water.*

No.	Stage.	Per Cent.	Time in Solution.	Time in Normal S. W.	Results.
869	1 cell.	10	3 hrs.	11½ hrs.	1–24 cells; many eggs broken; early cleavages irregular; scattered chromosomes, karyomeres, polyasters.

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* See note, p. 536.

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*This monumental and really epoch-making work was received too late to allow a proper reference to it in the preceding pages.

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XIV. EXPLANATION OF PLATES XLIII–LIX.

All figures represent whole eggs of *Crepidula plana*, fixed, stained, and mounted in balsam. They were drawn at stage level, with camera lucida, under Zeiss' Apochromatic Hom. Immersion Objective 3 mm., Comp. Ocular 4, and represent a magnification of 333 diameters. In the process of reproduction they have been reduced about one-fifth. The polar bodies are in all cases shaded by parallel lines, while the crenated line which appears in most of the figures represents, somewhat diagrammatically, the line of yolk spherules surrounding the cytoplasmic area. The spheres (centrospheres) are shown in dotted outline, as are also the cells and nuclei which lie at lower levels. Only abnormal eggs are represented, though normal eggs are found in many of the experiments.

Reference Letters.

A, B, C, D = Macromeres.
1a–1d = 1st set of micromeres.
2a–2d = 2d set of micromeres.
3a–3d = 3d set of micromeres.
4d = Mesentomere.
Am. = Amphiaster.
As. = Cytaster.
Ch. = Chromatin.
Ch. V. = Chromosomal vesicles.
End. = Endoderm.
F. C. = Follicle cells.
G. V. = Germinal vesicle.
L. = Lobe.
M. = Mesomere.
Memb. = Cell membrane.
N. = Nucleus.
Nl. = Nucleolus.
♀*N* = Egg nucleus.
♂*N* = Sperm nucleus.
Pb = Polar bodies.
PCp = Posterior cell plate.
S. = Sphere.
ShG = Shell gland.
S. G. = Sphere granules.
Sp. = Spindle.
V. = Velum.
Y. = Yolk.
Z. = Zwischenkörper.

PLATE XLIII.

Abnormalities Found in Nature.

Figs. 2–5, 9, 11–13, 15 probably show effects of pressure; figs. 6–8 and 16 and 17 probably show effects of diluted sea water.

Fig. 1. Immature egg probably incapable of maturation; follicle cells attached.

Fig. 2. First maturation division; abnormally large "yolk lobe" near vegetal pole, containing sperm nucleus.

Fig. 3. Enormous lobe at vegetal pole containing a sperm nucleus with sperm sphere attached; the small nucleus on the right is probably an accessory sperm nucleus; the granular body near the animal pole is probably the egg nucleus.

Fig. 4. Egg probably distorted by pressure; egg and sperm nuclei normal but removed from animal pole; abnormal lobe at vegetal pole.

Fig. 5. Egg with abnormally large yolk lobe.

Figs. 6–8. Eggs in which the 1st and 2d yolk cleavages were suppressed; two micromeres have been formed at the animal pole; karyomeres are present; probably the result of diluted sea water.

Fig. 9. Two-cell stage with entire amphiaster in one cell, and no nucleus or centrosome in the other cell. Probably spindle was displaced by pressure, to one side of cleavage plane; nevertheless egg has divided with formation of well marked "Zwischenkörper."

Fig. 10. Two cells; chromosomes scattered around active centers; this would probably give rise to karyomeres in the resting stages.

Fig. 11. Egg in which the 1st cleavage has been stopped and the nuclei, spheres and cytoplasm are out of their normal positions; probably the effect of pressure.

Figs. 12, 13. Eggs with large lobe opposite end of spindle, result of pressure. In fig. 12, the gonomeres are distinct; in fig. 13, the spindle has been pressed out of position and one of the nuclei lies in the cleavage plane and is constricted by it.

Fig. 14. Two-cell stage with gonomeres distinct.

Fig. 15. Two cells, interkinesis, with cell lobes in spindle axes of 2d cleavage.

Fig. 16. Two cells each with a tetraster.

Fig. 17. Four centrosomes, karyomeres, cleavage planes suppressed; the result, probably, of a tetraster.

PLATE XLIV.

Abnormalities Found in Nature.

Figs. 18–26. Show effects probably of pressure; figs. 27–29, of dilute sea water.

Fig. 18. Four-cell stage; the nucleus is entirely lacking in two of the cells, though sphere and "Zwischenkörper" are present. The lobe attached to these cells indicates that the egg was subjected to pressure at the time of the 1st cleavage; since cells do not divide when nuclei are not present it is probable that the nuclei were lost after the 2d cleavage, though there is no indication as to the manner of their disappearance.

Fig. 19. Four-cell stage, showing in the lobes the effects of pressure during the 2d cleavage.

Fig. 20. Third cleavage showing a lobe in the spindle axis of one cell, the result of pressure.

Fig. 21. Third cleavage spindles are present, three in one cell, one in the other. In the latter the spindle axis (*Sp4*) is normal, in the former abnormal; spindle one (*Sp1*) lies at a higher level than spindles two and three (*Sp2*, *Sp3*).

Fig. 22. Five macromeres, the two upper ones normal, the three lower ones abnormal; due to a tetraster in the lower cells.

Fig. 23. Seven macromeres, six of them reaching to the animal pole where they have formed six micromeres of the first set. One of the macromeres ($1D^2$) lies far from the animal pole and does not form a micromere. The nuclei are double or irregular in shape in macromeres $1B$ and $1D'$ and also in the micromeres derived from them.

Fig. 24. Seven macromeres, six of them reaching to the animal pole where they have formed six micromeres of the first set and are forming in lacotropic direction six micromeres of the second set; one micromere $1D^2$ lies at a deep level and forms no micromere; a triaster is present in $1B$, a double nucleus in $1D^2$ and double nuclei and spheres are found in the micromeres $1b$ and $1d$.

Fig. 25. Six macromeres ($2A$–$2D$), five of which are dividing in dexotropic direction to give rise to five micromeres of the third set. There are six micromeres of the second set and twelve of the first with a central cell containing two nuclei unaccounted for. The micromeres of the first set have divided unequally as in normal eggs.

Fig. 26. Three macromeres, one of them (*D*) giving rise to a micromere of the first set in normal direction; the other ($3B$, $3C$) have produced micromeres of the first, second and third sets in normal manner.

Figs. 27–29. Three embryos of the same laying showing the failure of the micromeres to overgrow the macromeres, probably the result of dilution of sea water with fresh water.

Fig. 27. Shows a gastrula with ectomeres and mesomeres (*M*) forming a cap on the entomeres, though in normal eggs the former would have overgrown the latter at this stage.

Fig. 28. View of right side of embryo showing shell gland (*ShG*), posterior cell plate (*PCp*) and velum (*V*) but with endoderm (*End*) protruding through blastopore.

Fig. 29. Embryo similar to the preceding but viewed from anterior pole.

PLATE XLV.

Cleavage of Isolated Blastomeres.

Fig. 30. Exp. 906: Third cleavage in $\frac{1}{2}$ or $\frac{2}{3}$ blastomeres, separated by pressure; the cells are smaller than the normal macromeres, owing probably to loss of yolk during pressure; each cell is dividing dexiotropically as it should in the third cleavage, but the relative positions of spindles and cytoplasmic areas in the two cells have undergone certain changes as may be seen by comparison with fig. 20.

Fig. 31. Exp. 921: $\frac{1}{2}$ or $\frac{2}{3}$ blastomeres, separated by shaking; the spindles are normal in position though division in *B* has been delayed.

Fig. 32. Exp. 855: $\frac{1}{4}$ blastomere, isolated by shaking; the first micromere has been formed and the second is forming in typical manner. Original animal pole indicated by polar bodies.

Fig. 33. Exp. 855: $\frac{1}{4}$ blastomere, isolated by shaking; the first and second micromeres have formed in normal manner, and the former is dividing as in a whole egg. Original animal pole indicated by polar body.

Fig. 34. No. 714: $\frac{2}{4}$ blastomeres, probably separated by pressure; each macromere has given off a micromere in dexiotropic direction, as in whole eggs.

Fig. 35. Exp. 855: $\frac{1}{2}$ or $\frac{2}{4}$ blastomeres, separated by shaking; each has produced a first micromere in dexiotropic and a second in læotropic direction, and the former are dividing in læotropic direction just as in whole eggs. The micromere plate is a continuous one, without breaks.

Fig. 36. No. 711: $\frac{3}{4}$ blastomeres, one macromere having been separated, probably by pressure. Each macromere has formed one micromere in normal fashion, but a gap exists between micromeres 1*c* and 1*a*.

Fig. 37. Exp. 864: $\frac{1}{2}$ or $\frac{2}{4}$ blastomeres, separated by hypertonic sea water and then left in normal sea water 10 hrs. Each macromere has formed three micromeres, and the first and second of these have subdivided in normal fashion. The egg is a whole in the sense only that it shows no gaps where cells are missing.

Fig. 38. No. 715: $\frac{1}{4}$ egg, probably separated by pressure after third cleavage, as shown by the fact that two micromeres of the first set (1*a* and 1*b*) are present.

Fig. 39. Exp. 875: $\frac{3}{4}$ blastomeres, macromere *C* having been destroyed in dilute sea water; the macromeres have given off the first micromeres, and these have subdivided in typical fashion, forming a triangular, but continuous micromere plate.

Fig. 40. Exp. 958: $\frac{3}{4}$ blastomeres, separated by shaking. Cells *A* and *D* have given rise to first and second micromeres in normal manner; *B* has formed only the first micromere and both 1*B* and 1*b* lack nuclei.

Fig. 41. Exp. 867: $\frac{3}{4}$ blastomeres, separated in hypertonic sea water. Typical cleavage of micromeres and macromeres of each quadrant represented, but the cells of one quadrant are wholly lacking.

Fig. 42. Exp. 1002 (2): $\frac{3}{4}$ blastomeres, separated by pressure. Cleavage typical for each quadrant, but delayed in quadrant *B*; the third micromere is just coming off from 2*D*.

Fig. 43. Exp. 958: $\frac{3}{4}$ blastomeres, separated by shaking. The cleavage of these three quadrants *A*, *C*, and *D* is absolutely typical; the cells of the fourth quadrant (*C*) are entirely lacking, but there are no gaps to mark the places from which they have dropped out. Each macromere has produced three micromeres, and in addition *D* has given rise to a fourth, the mesentoblast, $4d(= M^1, M^2)$. The micromeres have each divided in typical manner, giving rise to a cross (stippled cells) with three arms instead of four.

Fig. 44. Exp. 959: $\frac{3}{4}$ blastomeres separated by shaking; the cleavage of each macromere and the subdivisions of each micromere have taken place as in normal eggs.

PLATE XLVI.

Effects of Pressure.

Fig. 45. Exp. 918: Egg pressed during formation of second polar body, which is abnormally large.

Fig. 46. Exp. 918: Egg pressed during first cleavage which was rendered unequal; nuclei abnormally lobulated.

Fig. 47. Exp. 958: Egg shaken during first cleavage. Similar to preceding.

Fig. 48. Exp. 901: Very large yolk lobe at vegetal pole of one of the cells.

Fig. 49. No. 724: Egg probably pressed during the first cleavage; second cleavage spindles abnormally small.

Fig. 50. Exp. 918: Pressed during second cleavage in the direction of the spindle axes; macromeres *A* and *B* have divided normally giving rise to micromeres 1*a* and 1*b*; in macromere *CD* the second cleavage is still incomplete, and the entire amphiaster is abnormally large.

Fig. 51. Exp. 911: Pressed during the first cleavage; three macromeres with multiple nuclei formed probably as result of a triaster.

Fig. 52. Exp. 911: Pressed during second and third cleavages in chief axis of egg; macromeres *C* and *D* with their micromeres approximately normal; macromeres *A* and *B* have divided in abnormal planes giving rise to macromeres $1A^1$ and $1B^1$ instead of micromeres.

Fig. 53. Exp. 911: Pressed during second and third cleavages in chief axis of egg; all the upper tier of cells except 1*c* are abnormally large and are dividing like micromeres of the first set; in $1B^2$, 1*C* and $1D^2$ the direction of division is like that in the formation of the 2d set of micromeres.

FIG. 54. Exp. 915: Pressed during 2d and 3d cleavages in chief axis of egg; macromere A divided nearly equally into $1A^1$ and $1A^2$, which are now forming micromeres of the 1st set; macromeres $1B$, $1C$ and $1D$ are forming micromeres of the second set.

FIG. 55. Exp. 904: Pressed during 3d cleavage, the direction of which was changed from dexiotropic to læotropic in all quadrants except B; correspondingly the next cleavage (shown by spindles) is dexiotropic, instead of læotropic as in typical eggs.

FIG. 56. Exp. 915: Pressed in chief axis during the 4th cleavage, the chief result being the enlarged size of $2a$ and $2c$.

PLATE XLVII.

EFFECTS OF PRESSURE.

In all figures except 57, 65, 66 the axis of pressure was in the direction of the egg axis.

FIG. 57. Exp. 1003: Pressure parallel with the egg axis has produced a linear arrangement of the four macromeres, each of which preserves its original polarity and is dividing to form the first set of micromeres.

FIG. 58. Exp. 1003: Pressure in the chief axis of the egg has led to the formation of the micromeres of the 1st and 2d sets between the macromeres, instead of above them. The micromeres are larger than usual and the 1st set has subdivided giving off "turret" cells two of which ($1b^2$ and $1d^2$) are much larger than usual, while the other two ($1a^2$ and $1c^2$) have been forced to the lower side of the egg.

FIG. 59. Exp. 915: Pressure in the direction of the egg axis has led to the formation of eight macromeres, each of which is giving off in a dexiotropic direction a micromere of the 1st set.

FIG. 60. Exp. 915: Pressure in the egg axis during the 4th cleavage has caused the formation of larger micromeres than normal, especially in quadrants C and D, indeed the 2d division of C is nearly equal, giving rise to two macromeres; in the subdivisions of the 1st set of micromeres the "turret" cells ($1a^2$–$1d^2$) are much larger than usual.

FIG. 61. Exp. 915: In this case the pressure was probably applied after the formation of the 1st set of micromeres which are normal; the 2d set is also normal except in quadrant A, where the macromere $1A$ divided nearly equally into macromeres $2A^1$ and $2A^2$, and the former has divided into $2A^1$ and $2a^1$.

FIG. 62. Exp. 1001 (1): Normal except that macromere C divided equally at its first division; the right upper half then gave off a micromere of the first set ($1c^1$) which, judging by the shape of the cell, is about to form a "turret" cell as in the other three quadrants.

FIG. 63. Exp. 1004 (2): Pressed during the 3d cleavage, macromeres A and D divided nearly equally, thus increasing the number of macromeres to six, each of which has formed a micromere of the 1st set, while C has produced also a micromere of the 2d set ($2c$).

FIG. 64. Exp. 1003: Pressed during the formation of the 2d set of micromeres, which are much larger than usual; in the subdivision of $1b$ and $1c$ the peripheral products ("turret" cells) have been forced to the lower pole of the egg, and the macromeres have been pushed apart as in fig. 58.

FIG. 65. Exp. 915: Compressed obliquely to the egg axis; macromere B being shoved under the micromere plate; macromere A formed a first micromere larger than normal, which has divided equally ($1a^1$, $1a^2$), and then gave rise to a second "micromere," which is really a macromere ($2A^1$).

FIG. 66. Exp. 1001 (1): Compressed parallel with the egg axis, B and D being shoved under the other cells; the 1st and 2d sets of micromeres are nearly normal; at its 2d division A divided nearly equally into $3A$ and $3A^1$, each of which has formed a micromere ($2a$, $2a$) in a dexiotropic direction.

FIG. 67. Exp. 1001 (3): The cleavage is normal except that $4d$ is larger than usual, the result of pressure in the direction of the egg axis; the cells of the ectodermal cross are stippled.

PLATE XLVIII.

EFFECTS OF ELECTRIC CURRENT.

Eggs subjected to electric current (except figs. 76, 77) but probably showing effects of pressure.

FIG. 68. Exp. 1104 (?): 5 mil. amp., 5 min. First maturation spindle; the mitotic figure is much longer than normal, is central in the egg and the spindle fibres, centrosomes and astral rays are either lacking or very faint. The position is probably due to pressure.

FIG. 69. Exp. 1121 (2): 5 mil. amp. 10 min., normal 3½ hrs. Second cleavage spindles abnormal in position, first cleavage furrow incomplete, development stopped.

FIG. 70. Exp. 1121 (2): Similar to preceding.

FIG. 71. Exp. 1121 (2): Similar to preceding.

FIG. 72. Exp. 1121 (2): Similar to preceding.

FIG. 73. Exp. 1121 (2): Two cells, one containing a complete amphiaster but without any chromatin, the other containing two nuclei and one sphere, probably as the result of pressure at the close of the 1st cleavage.

FIG. 74. Exp. 1121 (2): Second cleavage spindle abnormal in position and division delayed in one blastomere.

FIG. 75. Exp. 1121 (2): Three macromeres in a linear series, the middle one containing two separate cytoplasmic areas, nuclei and spheres, the result of the suppression of the first cleavage, as in fig. 72.

FIG. 76. Exp. 1001 (3): A pressure experiment included in this plate by mistake, showing eight macromeres and eight micromeres.

FIG. 77. Exp. 919: Egg subjected to pressure, probably after formation of 1st set of micromeres. In quadrant *B* and *D* the 2d set of "micromeres" are really macromeres.

FIG. 78. Exp. 1110 (2): 5 mil. amp., 2 min., normal 22 hrs. Egg with scattered blastomeres in the stage of the formation of the 2d set of micromeres.

FIG. 79. Exp. 1121 (3): 5 mil. amp., 10 min., normal 16 hrs. Egg in stage of formation of 2d set of micromeres, showing effects of pressure.

PLATE XLIX.

EFFECTS OF ELECTRIC CURRENT.

FIG. 80. Exp. 997 (3): 1 volt, 15 min., normal 30 min. Egg and sperm nuclei are large and contain little chromatin, sphere material in granules, no segregation of yolk and cytoplasm.

FIG. 81. Exp. 997 (3): Similar to preceding.

FIG. 82. Exp. 1106: 5 mil. amp., 5 min., normal 17 hrs.; nuclear membrane dissolved and chromatin clumped; development stopped.

FIG. 83. Exp. 1106: Similar to preceding.

FIG. 84. Exp. 1106: Similar to preceding.

FIG. 85. Exp. 997 (3): Similar to fig. 80, but with nuclear membrane gone.

FIG. 86. Exp. 1140 (1): 2 mil. amp., 2 min., normal 5½ hrs. Chromatin disappearing.

FIG. 87. Exp. 1140 (2): 5 mil. amp., 10 min. Ordinary tetraster.

FIG. 88. Exp. 997 (2): 1 volt, 15 min., normal 2½ hrs. Plasma and nuclei displaced by convection current, as in centrifuged eggs.

FIG. 89. Exp. 997 (2): Similar to preceding.

FIG. 90. Exp. 1121 (2): Chromosomes have disappeared leaving the spindle fibers a little more chromatic than in normal eggs.

FIG. 91. Exp. 997 (2): 1 volt, 15 min., normal 2½ hrs. Chromatin largely dissolved and displaced toward lower pole; in right cell long strands of cytoplasm.

FIG. 92. Exp. 1121 (2): 5 mil. amp., 10 min., normal 3½ hrs. Spindle and chromosomes disappearing in left cell; others normal.

FIG. 93. Exp. 998 (2): 4 dry cells 1¾ hrs., normal 11 hrs. Evidently egg was in an advanced stage (ca. 42 cells) at the time of the experiment. Although the cells are not dead, the micromeres are rounded (framboisia) and many have dropped off.

PLATE L.

EFFECTS OF ABNORMAL TEMPERATURE.

FIG. 94. Exp. 1170 (1): Ca. 38° C. 1 hr.; egg irregular in outline, with archiplasm withdrawn into amphiaster, and into the surface layer. First maturation amphiaster irregular in shape and chromosomes scattered; sperm nucleus near vegetal pole.

FIG. 95. Exp. 1174 (2): 37° C. ½ hr., room temp. (27°) 3 hrs.; first polar body very large; chromosomes of second maturation division have formed karyomeres; sperm nucleus near animal pole.

FIG. 96. Exp. 1171 (1): Ca. 35° C. ½ hr.; 2-cell stage, showing dense aggregation of archiplasm around nuclei and spheres, with different kinds of cytoplasm in other parts of cell.

FIG. 97. Exp. 1171 (1): Similar to preceding; second cleavage spindles greatly modified; chromosomes scattered; archiplasm gathered in spindle areas and division wall.

FIG. 98. Exp. 1171 (2): Ca. 35° C. ½ hr., room temp. (ca. 24°–26°) 15 hrs.; the archiplasm has collected in central areas in each cell and in division walls; chromosomes are clumped and thrown out of cytoplasmic areas.

FIG. 99. Exp. 1171 (2): Similar to preceding.

FIG. 100. Exp. 962: On ice 16 hrs.; the spheres of the third cleavage are unusually distinct and the scattered sphere granules of the second cleavage (in the micromeres) are very large.

FIG. 101. Exp. 962: The spheres have a definite boundary, stain more deeply than usual and look almost like nuclei.

FIG. 102. Exp. 962: A later stage, with spheres similar to those shown in fig. 101.

FIG. 103. Exp. 964: On ice 40 hrs.; the sphere granules are especially large.

FIG. 104. Exp. 964: Similar to preceding.

FIG. 105. Exp. 964: Similar to preceding; many of the sphere granules are vesicular.

PLATE LI.

EFFECTS OF DECREASED OXYGEN TENSION.

FIGS. 106–109. Exp. 1010: Eggs placed for 36 hrs. in sea water which had been boiled to drive off contained gases, and then cooled; all development was completely stopped, but eggs were not killed; chromatin in the resting nuclei is collected into one or more masses; spindle fibres are distinct but astral rays are lacking; centrosomes and sphere granules are vesicular. The eggs used in this experiment were much smaller than normal, being only 120 μ in diameter.

FIGS. 110–111. Exp. 1025: Eggs left for 18 hrs. in a stoppered bottle of sea water, through which hydrogen had been run for 1 hr.; development completely stopped; nuclei and nucleoli large, little chromatin.

FIG. 112. Exp. 1016: Eggs placed for 27 hrs. in sea water which had been boiled and cooled; similar to preceding, development stopped.

FIG. 113. Exp. 1017: Eggs left for 48 hrs. in stoppered test tube of boiled and cooled sea water; development completely stopped; sphere granules prominent.

FIG. 114. Exp. 1016: Same as fig. 112; nuclei with little chromatin; sphere granules prominent.

FIG. 115. Exp. 1023: Eggs subjected to atmosphere of hydrogen for 2 hrs. and then left in open bottle for 2 hrs.; development stopped; eggs similar to all others subjected to decreased oxygen tension.

FIG. 116. Exp. 1017: Same as in fig. 113: The spindles are small, deep-staining and without astral rays; the chromosomes are arranged in a ring around the spindles.

FIGS. 117–118. Exp. 1025: Eggs left for 18 hrs. in stoppered bottle of sea water through which hydrogen had been run for 1 hr.; eggs similar to fig. 115; in fig. 118 there is an area of yolk (*Y*) around the upper poles of the spindles.

PLATE LII.

EFFECTS OF CARBONIC ACID.

Eggs in 1–8-cell stage left for 28 hrs. in sea water ⅔ saturated with CO_2.

FIG. 119. Exp. 1163: Egg showing the individually distinct chromosomal vesicles of the maturation divisions; also the cell membrane separated from the subjacent egg substance.

FIG. 120. Exp. 1163: Eggs showing five protoplasmic cells (one of them with four nuclei) on the unsegmented yolk.

FIG. 121. Exp. 1164: Side view of an egg similar to the preceding.

FIG. 122. Exp. 1163: The second polar body is abnormally large; an accessory aster (*S*) lies in the yolk; the division of the nucleus in the 1st cleavage has taken place normally, though the spheres are prevented from moving to their normal positions above the nuclei by the presence of the large polar body; the cleavage furrow cuts into the egg from the animal pole side only, and ends in a "cleavage head" as in cœlenterate eggs.

FIG. 123. Exp. 1163: The *C* and *D* quadrants are entirely normal; in the blastomere *AB*, the nucleus divided but the cell did not; these two nuclei in an undivided cell gave off a single blastomere of the first set (1*ab*) with large lobulated nucleus, the nuclei, spheres and cytoplasmic areas of the cell *AB* then came to lie at opposite sides of the macromere and each divided independently giving rise to a second micromere (2*a*, 2*b*) which is nearly normal.

FIG. 124. Exp. 1163: There are three macromeres one of which contains a perfect spindle but no chromatin; another contains a polyaster and has given off a large micromere with three nuclei; the third contains a single nucleus which is smaller than normal and has given off in reversed cleavage a 1st and a 2d micromere, the former of which is dividing.

FIG. 125. Exp. 1163: The first cleavage furrow failed to appear though the nuclei divided; at the 2d cleavage each nucleus with its adjacent oöplasm gave off a smaller macromere (*B* and *D*), leaving macromeres *A* and *C* still undivided; each of these four macromeres has given off a micromere of the 1st set.

FIG. 126. Exp. 1163: Three macromeres one of which contains several centrosomes and spheres (*S*) but no nucleus; another contains a bifurcated spindle and a normal one and has just given off two micromeres one on the right and one on the left; the other macromere contains a single nucleus and sphere and has just given off a micromere on the right; two large micromeres with abnormal nuclei occupy the center of the micromere field. There is a general resemblance of this egg to that shown in fig. 124.

FIG. 127. Exp. 1163: Side view of egg with two macromeres and several micromeres. Strands of protoplasm run from the polar bodies to the micromeres and from one of the latter to a macromere, suggesting the "spinning" activities of other eggs; lobes are also found on several cells.

FIG. 128. Exp. 1163: Two macromeres, each with two nuclei and two or more spheres; in the second cleavage the nuclei divided but the cell-body did not; in the third cleavage there was probably a triaster in each macromere, since only two micromeres of the first set were formed (1*ab*, 1*cd*) each with multiple nuclei; in the fourth cleavage each macromere contained two separate spindles and gave off two separate micromeres (2*a*, 2*b*, 2*c*, 2*d*) of the second set; the nuclei in the macromeres are so well separated that it is probable that at the next cleavage two independent spindles would form in each macromere and would lead to the formation of four micromeres of the third set.

FIG. 129. Exp. 1163: Macromeres *A* and *B* did not separate at the 2d cleavage, but each has given rise to three micromeres forming a typical micromere plate, though the direction of division in *A* and *B* has sometimes been atypical.

FIG. 130. Exp. 1164: Irregular cleavage mass in which it is not possible to identify many cells. Several of the cells show loose membranes and lobes.

FIG. 131. Exp. 1163: One of the macromeres (*D*) was separated from the other three, but each has given rise to three micromeres which have subdivided in normal manner, the micromeres formed from *D* lying on the right of the micromere plate formed from the other macromeres.

PLATE LIII.

EFFECTS OF DILUTED SEA WATER.

In figs. 132–135, 137–140, 144 the dilution was one part sea water to two parts fresh water; in all other cases the sea water was diluted with equal parts of fresh water. With higher dilutions the blastomeres tend to separate but do not swell appreciably.

FIG. 132. Exp. 875: Second polar spindle at animal pole; sperm nucleus has formed a spindle (σSp); the homogeneous chromatic sphere below this may represent an accessory sperm nucleus (σN).

FIG. 133. Exp. 875: First cleavage spindle; the seven chromatic spheres may represent accessory sperm nuclei (σN).

FIG. 134. Exp. 875: Enormous second polar body containing large nucleus and yolk; two nuclei and accessory sperm nucleus (σN) in egg.

FIG. 135. Exp. 875: Probably $\frac{1}{2}$ blastomere containing polyaster and with a micromere which has just divided.

FIG. 136. Exp. 872: Three blastomeres showing reversed polarity, the spheres, nuclei and cytoplasmic areas lying at the pole opposite the polar bodies; one sphere is found in each cell but in the two larger ones the nuclei are multiple.

FIG. 137. Exp. 875: Two macromeres, one containing a triaster, the other a tetraster; the two micromeres are normal except for their large size.

FIG. 138. Exp. 875: Similar to the preceding.

FIG. 139. Exp. 875: Side view of an egg similar to figs. 137, 138.

FIG. 140. Exp. 875: Macromeres A and B have not divided and the chromosomes are irregularly scattered in the spindle; macromeres C and D have divided normally giving rise to first and second micromeres and the first set are subdividing normally.

FIG. 141. Exp. 859: Chromosomes were scattered along the spindle during the third cleavage and have given rise to chromatic connections between daughter nuclei, which resemble amitoses.

FIG. 142. Exp. 872: The micromeres are larger than usual (two of them contain yolk) and they have caused a separation of A, B, from C, D.

FIG. 143. Exp. 872: The micromeres are larger than usual and contain yolk; the macromeres are separated and one which has just divided (Y, Y) contains yolk but no cytoplasmic areas; the chromosomes are here scattered along the spindle axis, thus forming a chromatic connection.

FIG. 144. Exp. 875: $\frac{2}{3}$ blastomeres, each of which has given rise to one micromere, which has subdivided; the macromeres contain spindles along which the chromosomes are scattered irregularly.

PLATE LIV.

EFFECTS OF DILUTED SEA WATER.

In all experiments represented on this plate sea water was diluted with equal parts of distilled water.

FIG. 145. Exp. 858: Several micromeres have been formed but the yolk has not divided; three of the cells contain several nuclei and spheres, the result probably of polyasters, and one contains a tetraster.

FIG. 146. Exp. 993 (1): Similar to the preceding; the protoplasmic micromeres are partly constricted from the yolk.

FIG. 147. Exp. 858: Exogastrula; similar to the preceding but of a more advanced stage; the multinucleate yolk cell is uncovered by the ectoderm.

FIG. 148. Exp. 993: Similar to the preceding.

FIG. 149. Exp. 993: Side view of an egg placed in diluted sea water in the 2-cell stage; the second cleavage of the yolk was suppressed, but several micromeres have been formed from each macromere.

FIG. 150. Exp. 956: Egg similar to the preceding, viewed from the animal pole; each macromere contains a large quadripartite nucleus and has given rise to twelve micromeres, which cannot be individually identified.

FIG. 151. Exp. 993: Isolated $\frac{1}{2}$ blastomere, the yolk cell has not divided, but contains several nuclei and has given rise to nine micromeres.

FIGS. 152, 153. Exp. 858: Top and side views of eggs which were placed in diluted sea water after formation of the 1st set of micromeres; several dividing cells contain triasters or tetrasters and the chromosomes are widely scattered; chromatic connections between daughter nuclei are falsely suggestive of amitosis.

FIG. 154. Exp. 858: Side view of egg placed in diluted sea water after formation of the three sets of micromeres which are approximately normal; scattered chromosomes have given rise to chromatic connections between daughter nuclei.

FIG. 155. Exp. 993: Isolated $\frac{2}{4}$ blastomeres which have produced a $\frac{2}{4}$ micromere plate; the macromere $4D$ has given off the mesentoblast $4d$ which is now dividing in normal manner.

FIG. 156. Exp. 871: The 1st set of micromeres have divided twice in normal directions, as indicated by the arrows, giving rise to twelve micromeres; in the formation of the 2d set of micromeres the division of the macromeres was approximately equal.

FIGS. 157, 158. Exp. 858: Eggs in which the nuclear division at the 2d cleavage took place normally, but in which the cell body did not divide; three quartets of micromeres were formed and have subdivided in approximately normal manner, although there are only two separate macromeres. The 4th quartet cells $4d$ and $4c$ form simultaneously from the undivided macromere CD, though in normal eggs $4d$ (the mesentoblast) forms at the 24-cell stage, while $4c$ (an entoblast) does not form until the 52-cell stage.

PLATE LV.

Effects of Hypertonic Sea Water.

All eggs shown on this plate are from Exp. 804, and were subjected to 1 per cent. NaCl in sea water for 4 hrs.

Figs. 159–163. The sperm nucleus lies in a small area of cytoplasm near the lower pole; the egg nucleus lies in a larger area of cytoplasm at the animal pole; various stages in the formation of the egg spindle are shown.

Fig. 164. Two spindles, probably those of egg and sperm, are joined at one pole.

Figs. 165, 166. Tetrasters, probably formed from the egg and the sperm spindles.

Fig. 167. Two spindles, probably those of the egg and the sperm, joined at one pole.

Fig. 168. Two spindles, probably those of the egg and sperm, quite separate.

Figs. 169, 170. Tetrasters in different phases of the separation of the chromosomes.

PLATE LVI.

Effects of Hypertonic Sea Water.

Fig. 171. Exp. 823: 3 per cent. NaCl, 16 hrs., normal 8 hrs. Egg nucleus very small and clear with chromosomes persistent within vesicle and egg centrosome outside vesicle; the sperm nucleus is enormous and contains much chromatic sap.

Fig. 172. Exp. 823: Similar to the preceding.

Fig. 173. Exp. 837: 1 per cent. KCl, 9 hrs., normal 35 hrs. Development has been stopped but the egg is not dead; the germ nuclei are very large and achromatic; small achromatic spherules lie in the cytoplasm.

Fig. 174. Exp. 822: 2 per cent. NaCl, 16 hrs., normal 8 hrs. Achromatin in large and small vesicles in the cytoplasm; germ nuclei normal.

Fig. 175. Exp. 822: The germ nuclei are broken up into many separate vesicles, each with a chromatic nucleolus.

Fig. 176. Exp. 823: Two-cell stage from same experiment as figs. 171, 172; nuclei very large and achromatic.

Fig. 177. Exp. 822: Side view of egg in 2-cell stage, each cell containing a large sphere (*S*) and one or more chromatic and two or three achromatic nuclear vesicles.

Fig. 178, 179. Exp. 810: 3 per cent. NaCl, 15 hrs.: Side views of egg in 2-cell stage showing the chromatin massed within the nuclear vesicle; the latter are elongated along the line of the former spindle axis.

Figs. 180–182. Same experiment as preceding; 4-cell stages from animal pole.

Fig. 180. In one half of egg the remains of the 2d cleavage spindle are still visible and the chromatin has formed no nuclear vesicle; in the other half the nuclear vesicles are elongated along the line of the spindle axis, the chromatin being massed at the ends of the vesicle nearest the spheres.

Fig. 181. Nuclear vesicles elongated along the spindle axis are present in three of the cells, and in each the chromatin is massed at the end of the vesicle nearest the sphere; in the fourth cell no nuclear vesicle is present, but traces of spindle fibres may be seen.

Fig. 182. In all four cells the nuclear vesicles are rounded and the chromatin is massed near the center of each vesicle.

PLATE LVII.

Effects of Hypertonic Sea Water.

Figs. 183–185 were in 1-cell stage at beginning of experiment; Figs. 186–196 were in 2-cell stage.

Fig. 183. Exp. 805: 2 per cent. NaCl, 4 hrs.; in both egg and sperm nuclei the chromatin is aggregated into a dense mass in the center of the nuclear vesicle; there are many cytasters near the sperm nucleus.

Fig. 184. Exp. 809: 2 per cent. NaCl, 15 hrs.; the chromatic and achromatic parts of the germ nuclei are in separate vesicles.

Fig. 185. Exp. 809: Similar to the preceding; a double cytaster is present near the germ nucleus.

Fig. 186. Exp. 809: 2 per cent. NaCl, 15 hrs.; the chromatic and achromatic parts of the nucleus are in separate vesicles; the achromatic vesicles are numerous and scattered.

Fig. 187. Exp. 809: Side view of egg similar to preceding.

Fig. 188. Exp. 809: Egg similar to the preceding showing two principal achromatic vesicles (*Ach*) and many smaller vesicles or sphere granules.

Figs. 189, 190. Exp. 805: 2 per cent. NaCl, 4 hrs.; the 2d cleavage spindles are much shrunken in size; the archiplasm around them is much condensed while many small radiating masses of archiplasm are left along the astral radiations as cytasters.

Figs. 191, 192. Exp. 823: 3 per cent. NaCl, 16 hrs., normal 8 hrs.; top and side views of eggs showing in addition to the principal masses of chromatin (*Ch*) very numerous granules or spherules which probably represent scattered achromatic material (*Ach*).

Figs. 193, 194. Exp. 837: 1 per cent. KCl, 9 hrs., normal 36 hrs.; the numerous karyomeres have begun to absorb achromatin and to fuse together.

FIGS. 195, 196. Exp. 808: 1 per cent. NaCl, 15 hrs.; top and side views of eggs with two macromeres and two micromeres, each with many masses of chromatin (karyomeres) and several spheres or asters.

PLATE LVIII.

EFFECTS OF HYPERTONIC SEA WATER.

All eggs represented on this plate were put into hypertonic solutions in the 1-cell stage, and with the exception of fig. 197 none of them show division of the yolk.

FIG. 197. Exp. 842: 4 per cent. $MgCl_2$, 5 hrs., normal 17 hrs.; the two cells at the upper pole are probably enormous polar bodies, both of which contain mitotic figures.

FIG. 198. Exp. 991: 1 per cent. $MgCl_2$, 18 hrs.; one large cell (macromere) contains all the yolk, two smaller cells (micromeres) are purely protoplasmic; both macromeres and micromeres contain many nuclei (karyomeres), the chromatin of each being condensed into one or two masses.

FIG. 199. Exp. 834: 3 per cent. $MgCl_2$, 9 hrs., normal 9 hrs.; both macromere and micromere contain mitotic figures with many poles and scattered chromosomes; sphere granules at the animal pole of the micromere.

FIG. 200. Exp. 833: 2 per cent. $MgCl_2$, 9 hrs., normal 9 hrs.; resting stage following a division stage like that of the preceding figure.

FIG. 201. Exp. 838: Herbst's Ca-free sea water, 18 hrs.; micromere and macromere containing many nuclei and cytasters.

FIG. 202. Exp. 825: 2 per cent. NaCl, 16 hrs., normal 23 hrs.; two spheres and many scattered nuclei (karyomeres) in both macromere and micromere.

FIG. 203. Exp. 827: $\frac{3}{4}$ per cent. NaCl, 9 hrs., normal 8 hrs.; many asters and scattered chromosomes in the macromere and in one of the micromeres.

FIG. 204. Exp. 863: 2 per cent. NaCl, $2\frac{1}{2}$ hrs., normal 10 hrs.; similar to the preceding; scattered spindles and chromosomes; three small micromeres (lobes) budding off from the macromere; sphere granules at the animal pole of the micromeres.

FIG. 205. Exp. 843: 1 per cent. NaCl, 6 hrs., normal 15 hrs.; several micromeres, three of them containing several nuclei or groups of chromosomes; the macromere contains many nuclei (karyomeres).

FIG. 206. Exp. 827: $\frac{3}{4}$ per cent. NaCl, 9 hrs., normal 8 hrs.; macromere and micromeres containing many karyomeres and asters.

FIG. 207. Exp. 830: 2 per cent. NaCl, 9 hrs., normal 32 hrs.; macromeres and micromeres containing a large number of karyomeres or asters; sphere granules under the polar body.

FIG. 208. Exp. 833: 2 per cent. $MgCl_2$, 9 hrs., normal 9 hrs.; many nuclei (karyomeres) and spheres in each cell.

PLATE LIX.

EFFECTS OF HYPERTONIC SEA WATER.

Eggs represented in figs. 209–218 were in the 2-cell stage when the experiments began, and in none of them did the yolk undergo any subsequent cleavage; figs. 219–223 were in the 4-cell stage at the beginning of the experiment.

FIG. 209. Exp. 865: 4 per cent. NaCl, 2 hrs., normal 10 hrs.; the nuclear division at the 2d cleavage occurred regularly but the cell division was suppressed, thus leaving two nuclei of normal appearance, in each cell; at least three spheres are present in the left cell.

FIG. 210. Exp. 804: 1 per cent. NaCl, 4 hrs.; partial suppression of the 2d cleavage furrow which is limited to a shallow furrow on the animal pole side of the egg; the nuclei are irregular and densely chromatic.

FIG. 211. Exp. 864: 3 per cent. NaCl, 3 hrs., normal 10 hrs.; the 2d cleavage spindles have the position of the 3d cleavage spindles of normal eggs; the chromosomes are scattered abnormally on the spindles.

FIG. 212. Exp. 865: 4 per cent. NaCl, 2 hrs., normal 10 hrs.; the two macromeres have each given off a first and a second micromere, the first by dexiotropic and the second by lævotropic division, as in normal eggs. Each of the first micromeres contain a single nucleus and sphere; the second micromeres and the macromeres contain several spheres and karyomeres or groups of chromosomes.

FIG. 213. Exp. 863: 2 per cent. NaCl, $2\frac{1}{2}$ hrs., normal 10 hrs.; the first set of micromeres have been formed in dexiotropic direction, as in normal cleavage; the macromeres are dividing again with double spindles or tetrasters.

FIG. 214. Exp. 846: 4 per cent. $MgCl_2$, 4 hrs., normal 16 hrs.; the two macromeres are separated by a group of micromeres which have formed along both sides of the 1st cleavage plane; the polarity of each half thus appears to have been changed, the centers of the ectodermal pole being the surface of contact between the two halves; this may be due, in part, to the outward rotation of the macromeres at the vegetal pole and their inward rotation at the animal pole.

FIG. 215. Exp. 846: 4 per cent. $MgCl_2$, 4 hrs., normal 16 hrs.; side view of an egg with two macromeres and six micromeres; the two original micromeres of the first set have subdivided as indicated by the arrows, the other two micromeres are of the 2d set; all the micromeres contain karyomeres; the macromeres contain chromosomes scattered apparently, along the line of the 1st cleavage spindle.

FIG. 216. Exp. 846: Egg from same experiment as the preceding; the nuclei at the vegetal pole are probably derived from chromosomes which were scattered along the first cleavage spindle as in fig. 215.

FIG. 217. Exp. 822: 2 per cent. NaCl, 16 hrs., normal 8 hrs.; irregular and unequal 2d cleavage with several karyomeres of varying size in each cell.

FIG. 218. Exp. 842: 4 per cent. $MgCl_2$, 5 hrs., normal 17 hrs.; the divisions of the cell body at the 2d cleavage were suppressed in the left half, but two micromeres are arising in normal manner from this half; several accessory asters are present in this half which have served to scatter the chromosomes. The right half of the egg is quite normal.

FIG. 219. Exp. 867: 8 per cent. $MgCl_2$, $\frac{3}{4}$ hr., normal $6\frac{1}{2}$ hrs.; the 3d cleavage was very irregular and has given rise to a large number of karyomeres, and spheres which are in division in three quadrants of the egg.

FIG. 220. Exp. 972: 2 per cent. NaCl, 16 hrs., normal 24 hrs.; second quartet formation; karyomeres or polyasters in each of the cells.

FIG. 221. Exp. 814: 2 per cent. NaCl, 1 hr., normal 17 hrs.; second quartet formation; karyomeres and spheres in every cell.

FIG. 222. Exp. 828: 1 per cent. NaCl, 2 hrs., normal $6\frac{1}{2}$ hrs.; second quartet formation; karyomeres and polyasters in every cell.

FIG. 223. Exp. 972: 2 per cent. NaCl, 16 hrs., normal 24 hrs.; karyomeres (and asters) in every cell; all nuclei are vesicular and contain achromatin as well as chromatin.

PLATE XLIII.

Abnormalities Found in Nature.

Figs. 2–5, 9, 11–13, 15 probably show effects of pressure; figs. 6–8 and 16 and 17 probably show effects of diluted sea water.

Fig. 1. Immature egg probably incapable of maturation; follicle cells attached.

Fig. 2. First maturation division; abnormally large "yolk lobe" near vegetal pole, containing sperm nucleus.

Fig. 3. Enormous lobe at vegetal pole containing a sperm nucleus with sperm sphere attached; the small nucleus on the right is probably an accessory sperm nucleus; the granular body near the animal pole is probably the egg nucleus.

Fig. 4. Egg probably distorted by pressure; egg and sperm nuclei normal but removed from animal pole; abnormal lobe at vegetal pole.

Fig. 5. Egg with abnormally large yolk lobe.

Figs. 6–8. Eggs in which the 1st and 2d yolk cleavages were suppressed; two micromeres have been formed at the animal pole; karyomeres are present; probably the result of diluted sea water.

Fig. 9. Two-cell stage with entire amphiaster in one cell, and no nucleus or centrosome in the other cell. Probably spindle was displaced by pressure, to one side of cleavage plane; nevertheless egg has divided with formation of well marked "Zwischenkörper."

Fig. 10. Two cells; chromosomes scattered around active centers; this would probably give rise to karyomeres in the resting stages.

Fig. 11. Egg in which the 1st cleavage has been stopped and the nuclei, spheres and cytoplasm are out of their normal positions; probably the effect of pressure.

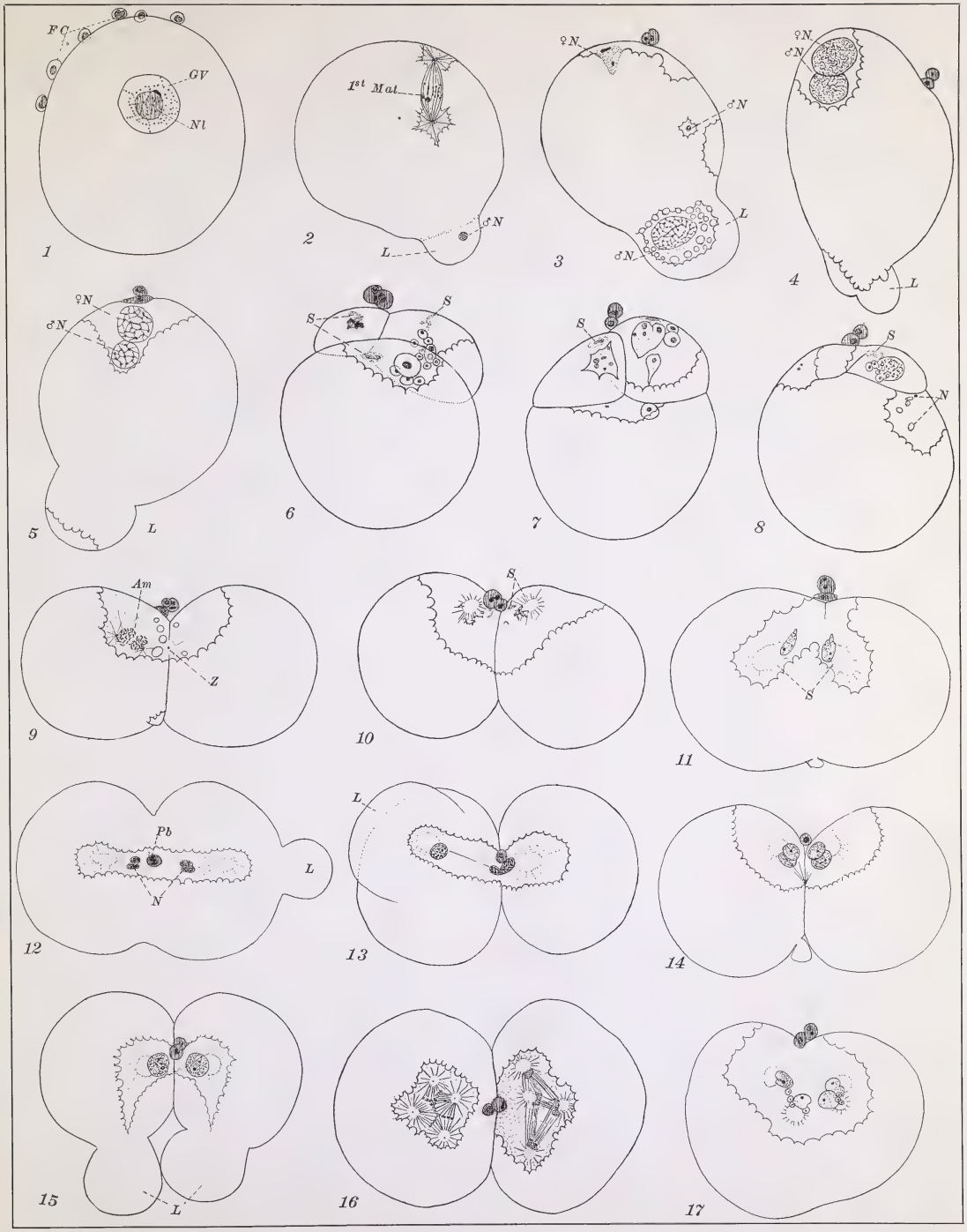
Figs. 12, 13. Eggs with large lobe opposite end of spindle, result of pressure. In fig. 12, the gonomeres are distinct; in fig. 13, the spindle has been pressed out of position and one of the nuclei lies in the cleavage plane and is constricted by it.

Fig. 14. Two-cell stage with gonomeres distinct.

Fig. 15. Two cells, interkinesis, with cell lobes in spindle axes of 2d cleavage.

Fig. 16. Two cells each with a tetraster.

Fig. 17. Four centrosomes, karyomeres, cleavage planes suppressed; the result, probably, of a tetraster.



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CONKLIN: NUCLEAR AND CELL DIVISION IN CREPIDULA

ABNORMALITIES FOUND IN NATURE

PLATE XLIV.

Abnormalities Found in Nature.

Figs. 18–26. Show effects probably of pressure; figs. 27–29, of dilute sea water.

Fig. 18. Four-cell stage; the nucleus is entirely lacking in two of the cells, though sphere and "Zwischenkörper" are present. The lobe attached to these cells indicates that the egg was subjected to pressure at the time of the 1st cleavage; since cells do not divide when nuclei are not present it is probable that the nuclei were lost after the 2d cleavage, though there is no indication as to the manner of their disappearance.

Fig. 19. Four-cell stage, showing in the lobes the effects of pressure during the 2d cleavage.

Fig. 20. Third cleavage showing a lobe in the spindle axis of one cell, the result of pressure.

Fig. 21. Third cleavage spindles are present, three in one cell, one in the other. In the latter the spindle axis (*Sp*4) is normal, in the former abnormal; spindle one (*Sp*1) lies at a higher level than spindles two and three (*Sp*2, *Sp*3).

Fig. 22. Five macromeres, the two upper ones normal, the three lower ones abnormal; due to a tetraster in the lower cells.

Fig. 23. Seven macromeres, six of them reaching to the animal pole where they have formed six micromeres of the first set. One of the macromeres ($1D^2$) lies far from the animal pole and does not form a micromere. The nuclei are double or irregular in shape in macromeres 1*B* and 1*D'* and also in the micromeres derived from them.

Fig. 24. Seven macromeres, six of them reaching to the animal pole where they have formed six micromeres of the first set and are forming in lacotropic direction six micromeres of the second set; one micromere $1D^2$ lies at a deep level and forms no micromere; a triaster is present in 1*B*, a double nucleus in $1D^2$ and double nuclei and spheres are found in the micromeres 1*b* and 1*d*.

Fig. 25. Six macromeres (2*A*–2*D*), five of which are dividing in dexotropic direction to give rise to five micromeres of the third set. There are six micromeres of the second set and twelve of the first with a central cell containing two nuclei unaccounted for. The micromeres of the first set have divided unequally as in normal eggs.

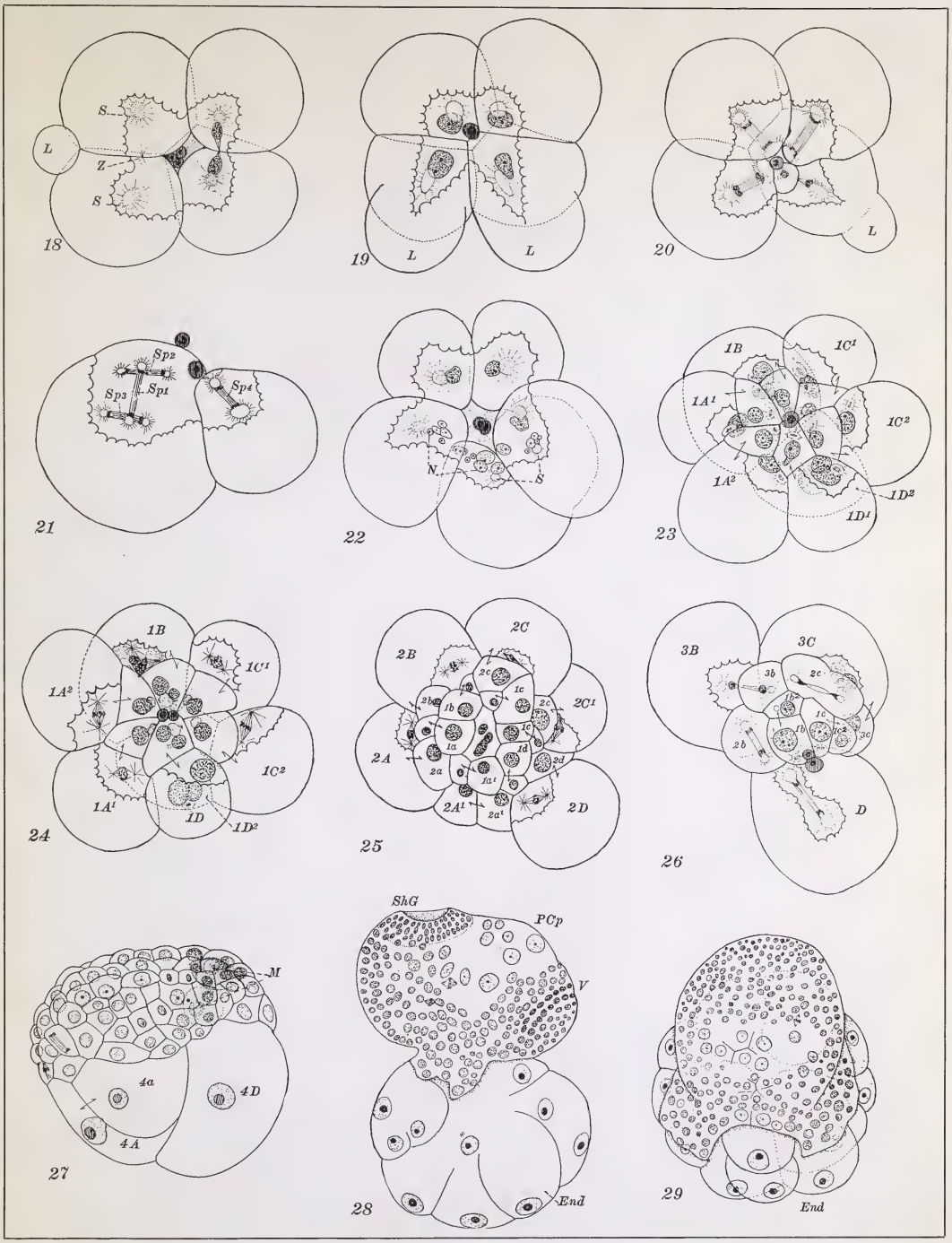
Fig. 26. Three macromeres, one of them (*D*) giving rise to a micromere of the first set in normal direction; the other (3*B*, 3*C*) have produced micromeres of the first, second and third sets in normal manner.

Figs. 27–29. Three embryos of the same laying showing the failure of the micromeres to overgrow the macromeres, probably the result of dilution of sea water with fresh water.

Fig. 27. Shows a gastrula with ectomeres and mesomeres (*M*) forming a cap on the entomeres, though in normal eggs the former would have overgrown the latter at this stage.

Fig. 28. View of right side of embryo showing shell gland (*ShG*), posterior cell plate (*PCp*) and velum (*V*) but with endoderm (*End*) protruding through blastopore.

Fig. 29. Embryo similar to the preceding but viewed from anterior pole.



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CONKLIN: NUCLEAR AND CELL DIVISION IN CREPIDULA

ABNORMALITIES FOUND IN NATURE

PLATE XLV.

Cleavage of Isolated Blastomeres.

Fig. 30. Exp. 906: Third cleavage in $\frac{1}{2}$ or $\frac{2}{4}$ blastomeres, separated by pressure; the cells are smaller than the normal macromeres, owing probably to loss of yolk during pressure; each cell is dividing dexiotropically as it should in the third cleavage, but the relative positions of spindles and cytoplasmic areas in the two cells have undergone certain changes as may be seen by comparison with fig. 20.

Fig. 31. Exp. 921: $\frac{1}{2}$ or $\frac{2}{4}$ blastomeres, separated by shaking; the spindles are normal in position though division in *B* has been delayed.

Fig. 32. Exp. 855: $\frac{1}{4}$ blastomere, isolated by shaking; the first micromere has been formed and the second is forming in typical manner. Original animal pole indicated by polar bodies.

Fig. 33. Exp. 855: $\frac{1}{4}$ blastomere, isolated by shaking; the first and second micromeres have formed in normal manner, and the former is dividing as in a whole egg. Original animal pole indicated by polar body.

Fig. 34. No. 714: $\frac{2}{4}$ blastomeres, probably separated by pressure; each macromere has given off a micromere in dexiotropic direction, as in whole eggs.

Fig. 35. Exp. 855: $\frac{1}{2}$ or $\frac{2}{4}$ blastomeres, separated by shaking; each has produced a first micromere in dexiotropic and a second in læotropic direction, and the former are dividing in læotropic direction just as in whole eggs. The micromere plate is a continuous one, without breaks.

Fig. 36. No. 711: $\frac{3}{4}$ blastomeres, one macromere having been separated, probably by pressure. Each macromere has formed one micromere in normal fashion, but a gap exists between micromeres 1*c* and 1*a*.

Fig. 37. Exp. 864: $\frac{1}{2}$ or $\frac{2}{4}$ blastomeres, separated by hypertonic sea water and then left in normal sea water 10 hrs. Each macromere has formed three micromeres, and the first and second of these have subdivided in normal fashion. The egg is a whole in the sense only that it shows no gaps where cells are missing.

Fig. 38. No. 715: $\frac{1}{4}$ egg, probably separated by pressure after third cleavage, as shown by the fact that two micromeres of the first set (1*a* and 1*b*) are present.

Fig. 39. Exp. 875: $\frac{3}{4}$ blastomeres, macromere *C* having been destroyed in dilute sea water; the macromeres have given off the first micromeres, and these have subdivided in typical fashion, forming a triangular, but continuous micromere plate.

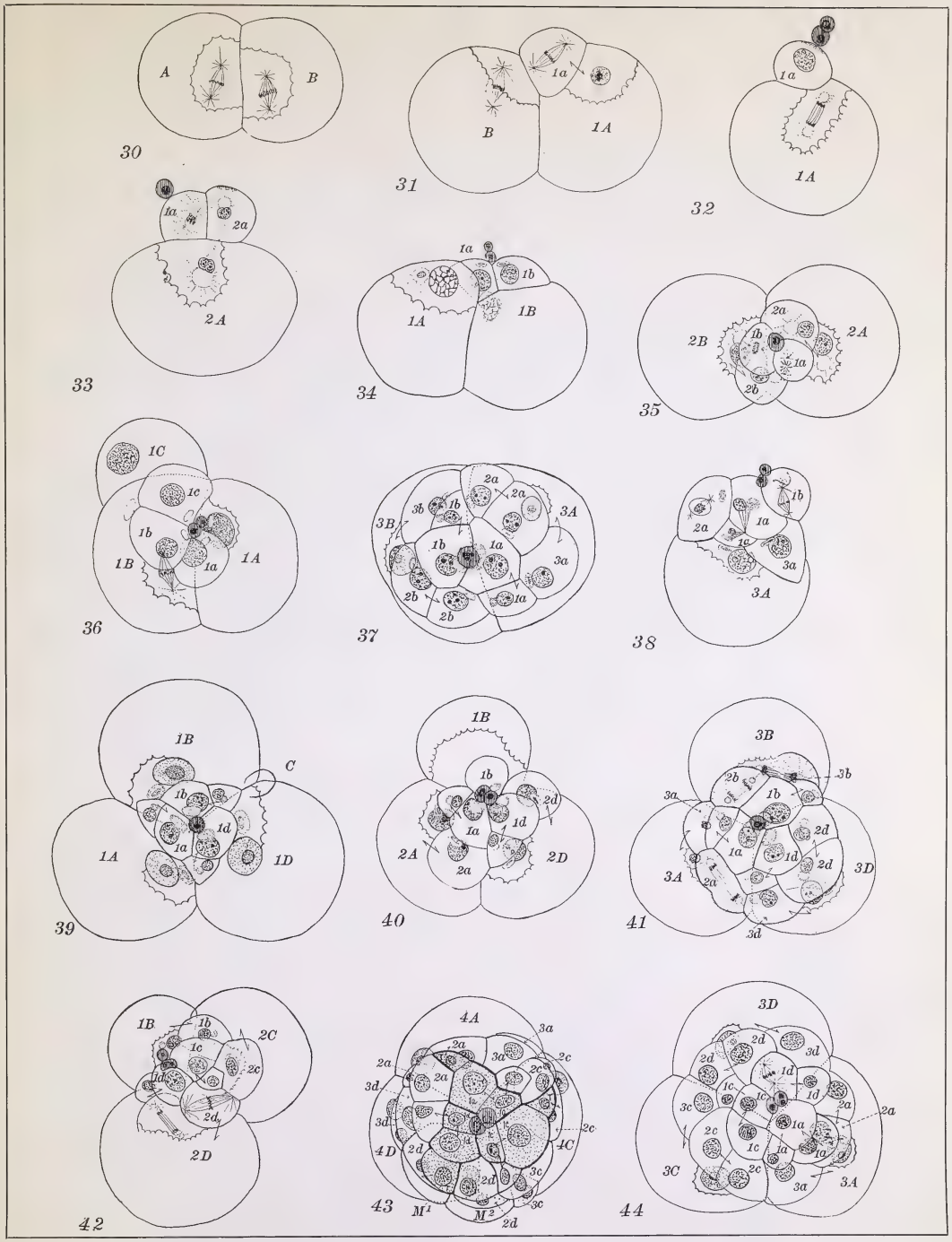
Fig. 40. Exp. 958: $\frac{3}{4}$ blastomeres, separated by shaking. Cells *A* and *D* have given rise to first and second micromeres in normal manner; *B* has formed only the first micromere and both 1*B* and 1*b* lack nuclei.

Fig. 41. Exp. 867: $\frac{3}{4}$ blastomeres, separated in hypertonic sea water. Typical cleavage of micromeres and macromeres of each quadrant represented, but the cells of one quadrant are wholly lacking.

Fig. 42. Exp. 1002 (2): $\frac{3}{4}$ blastomeres, separated by pressure. Cleavage typical for each quadrant, but delayed in quadrant *B*; the third micromere is just coming off from 2*D*.

Fig. 43. Exp. 958: $\frac{3}{4}$ blastomeres, separated by shaking. The cleavage of these three quadrants *A*, *C*, and *D* is absolutely typical; the cells of the fourth quadrant (*C*) are entirely lacking, but there are no gaps to mark the places from which they have dropped out. Each macromere has produced three micromeres, and in addition *D* has given rise to a fourth, the mesentoblast, $4d(= M^1, M^2)$. The micromeres have each divided in typical manner, giving rise to a cross (stippled cells) with three arms instead of four.

Fig. 44. Exp. 959: $\frac{3}{4}$ blastomeres separated by shaking; the cleavage of each macromere and the subdivisions of each micromere have taken place as in normal eggs.



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CONKLIN: NUCLEAR AND CELL DIVISION IN CREPIDULA

CLEAVAGE OF ISOLATED BLASTOMERES

PLATE XLVI.

Effects of Pressure.

Fig. 45. Exp. 918: Egg pressed during formation of second polar body, which is abnormally large.

Fig. 46. Exp. 918: Egg pressed during first cleavage which was rendered unequal; nuclei abnormally lobulated.

Fig. 47. Exp. 958: Egg shaken during first cleavage. Similar to preceding.

Fig. 48. Exp. 901: Very large yolk lobe at vegetal pole of one of the cells.

Fig. 49. No. 724: Egg probably pressed during the first cleavage; second cleavage spindles abnormally small.

Fig. 50. Exp. 918: Pressed during second cleavage in the direction of the spindle axes; macromeres *A* and *B* have divided normally giving rise to micromeres 1*a* and 1*b*; in macromere *CD* the second cleavage is still incomplete, and the entire amphiaster is abnormally large.

Fig. 51. Exp. 911: Pressed during the first cleavage; three macromeres with multiple nuclei formed probably as result of a triaster.

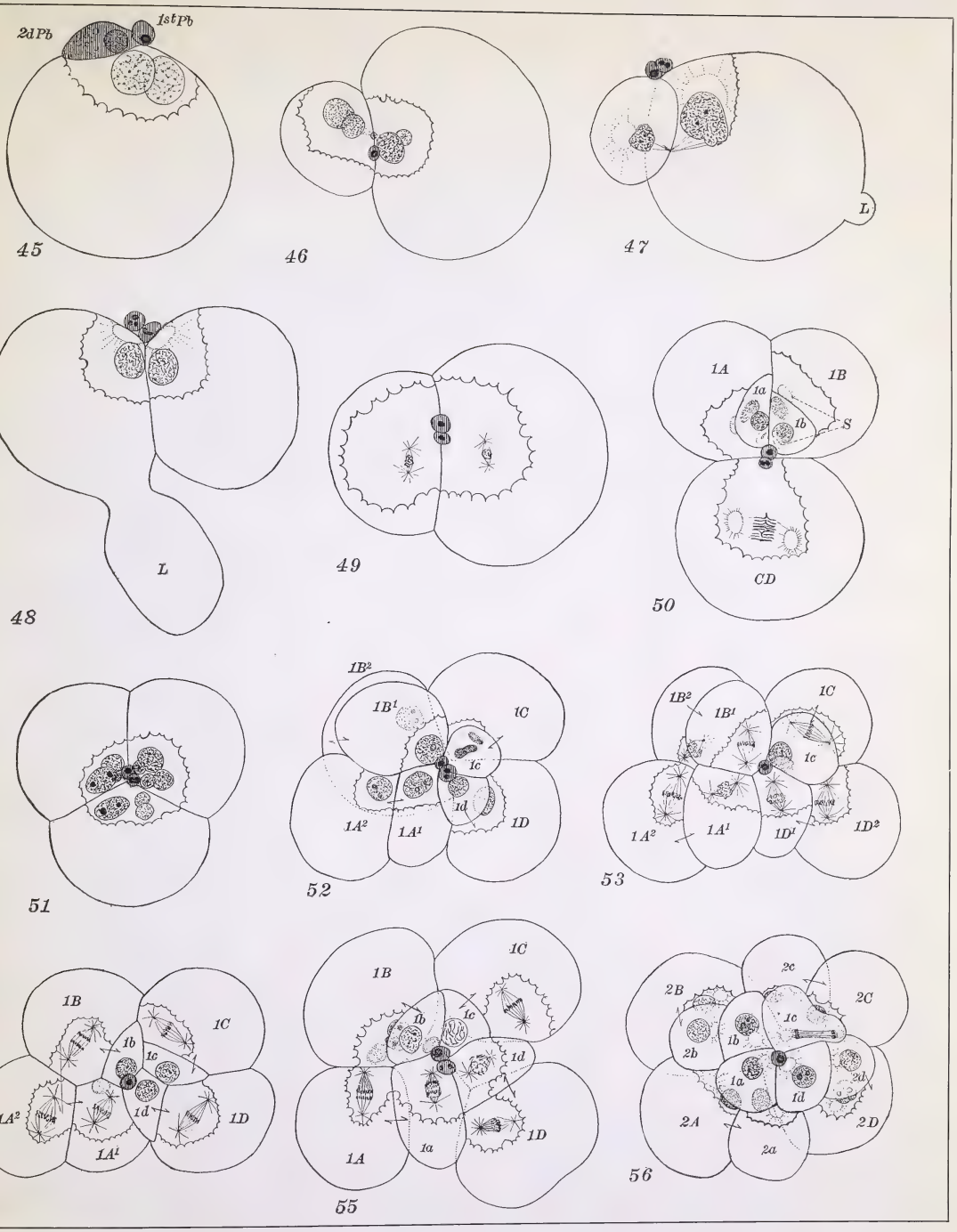
Fig. 52. Exp. 911: Pressed during second and third cleavages in chief axis of egg; macromeres *C* and *D* with their micromeres approximately normal; macromeres *A* and *B* have divided in abnormal planes giving rise to macromeres $1A^1$ and $1B^1$ instead of micromeres.

Fig. 53. Exp. 911: Pressed during second and third cleavages in chief axis of egg; all the upper tier of cells except 1*c* are abnormally large and are dividing like micromeres of the first set; in $1B^2$, 1*C* and $1D^2$ the direction of division is like that in the formation of the 2d set of micromeres.

Fig. 54. Exp. 915: Pressed during 2d and 3d cleavages in chief axis of egg; macromere *A* divided nearly equally into $1A^1$ and $1A^2$, which are now forming micromeres of the 1st set; macromeres 1*B*, 1*C* and 1*D* are forming micromeres of the second set.

Fig. 55. Exp. 904: Pressed during 3d cleavage, the direction of which was changed from dexiotropic to læotropic in all quadrants except *B*; correspondingly the next cleavage (shown by spindles) is dexiotropic, instead of læotropic as in typical eggs.

Fig. 56. Exp. 915: Pressed in chief axis during the 4th cleavage, the chief result being the enlarged size of 2*a* and 2*c*.



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CONKLIN: NUCLEAR AND CELL DIVISION IN CREPIDULA

EFFECTS OF PRESSURE

PLATE XLVII.

Effects of Pressure.

In all figures except 57, 65, 66 the axis of pressure was in the direction of the egg axis.

Fig. 57. Exp. 1003: Pressure parallel with the egg axis has produced a linear arrangement of the four macromeres, each of which preserves its original polarity and is dividing to form the first set of micromeres.

Fig. 58. Exp. 1003: Pressure in the chief axis of the egg has led to the formation of the micromeres of the 1st and 2d sets between the macromeres, instead of above them. The micromeres are larger than usual and the 1st set has subdivided giving off "turret" cells two of which ($1b^2$ and $1d^2$) are much larger than usual, while the other two ($1a^2$ and $1c^2$) have been forced to the lower side of the egg.

Fig. 59. Exp. 915: Pressure in the direction of the egg axis has led to the formation of eight macromeres, each of which is giving off in a dexiotropic direction a micromere of the 1st set.

Fig. 60. Exp. 915: Pressure in the egg axis during the 4th cleavage has caused the formation of larger micromeres than normal, especially in quadrants *C* and *D*, indeed the 2d division of *C* is nearly equal, giving rise to two macromeres; in the subdivisions of the 1st set of micromeres the "turret" cells ($1a^2$–$1d^2$) are much larger than usual.

Fig. 61. Exp. 915: In this case the pressure was probably applied after the formation of the 1st set of micromeres which are normal; the 2d set is also normal except in quadrant *A*, where the macromere 1*A* divided nearly equally into macromeres $2A^1$ and $2A^2$, and the former has divided into $2A^1$ and $2a^1$.

Fig. 62. Exp. 1001 (1): Normal except that macromere *C* divided equally at its fisrt division; the right upper half then gave off a micromere of the first set ($1c^1$) which, judging by the shape of the cell, is about to form a "turret" cell as in the other three quadrants.

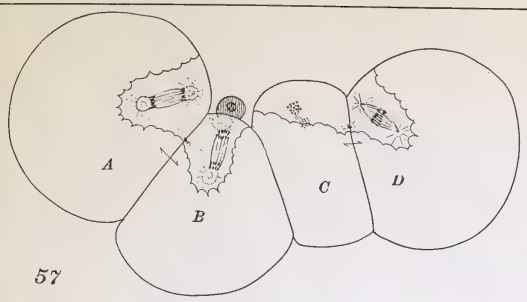
Fig. 63. Exp. 1004 (2): Pressed during the 3d cleavage, macromeres *A* and *D* divided nearly equally, thus increasing the number of macromeres to six, each of which has formed a micromere of the 1st set, while *C* has produced also a micromere of the 2d set (2*c*).

Fig. 64. Exp. 1003: Pressed during the formation of the 2d set of micromeres, which are much larger than usual; in the subdivision of 1*b* and 1*c* the peripheral products ("turret" cells) have been forced to the lower pole of the egg, and the macromeres have been pushed apart as in fig. 58.

Fig. 65. Exp. 915: Compressed obliquely to the egg axis; macromere *B* being shoved under the micromere plate; macromere *A* formed a first micromere larger than normal, which has divided equally ($1a^1$, $1a^2$), and then gave rise to a second "micromere," which is really a macromere ($2A^1$).

Fig. 66. Exp. 1001 (1): Compressed parallel with the egg axis, *B* and *D* being shoved under the other cells; the 1st and 2d sets of micromeres are nearly normal; at its 2d division *A* divided nearly equally into 3*A* and $3A^1$, each of which has formed a micromere (2*a*, 2*a*) in a dexiotropic direction.

Fig. 67. Exp. 1001 (3): The cleavage is normal except that 4*d* is larger than usual, the result of pressure in the direction of the egg axis; the cells of the ectodermal cross are stippled.



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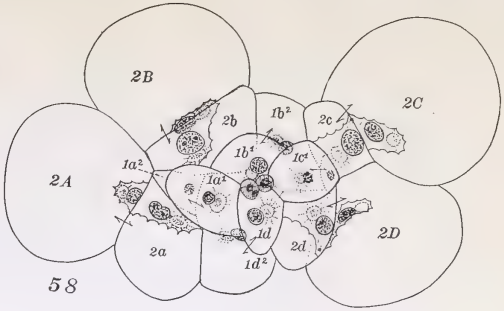

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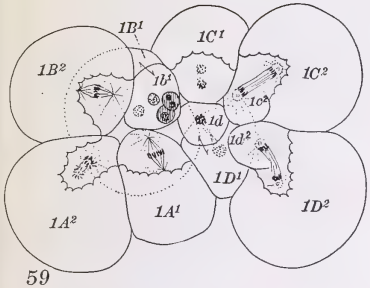

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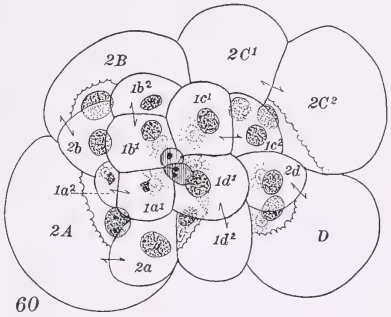

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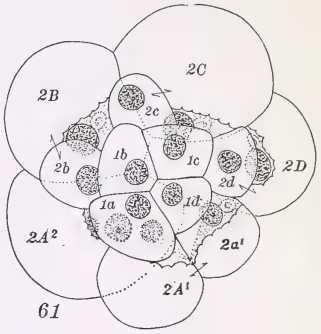

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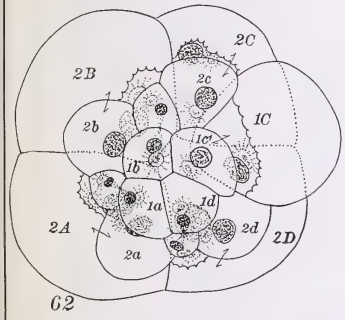

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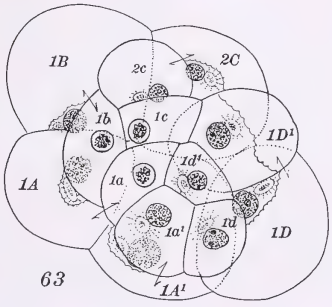

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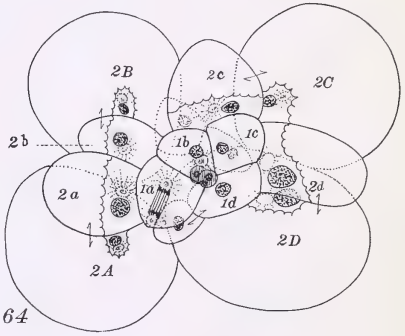

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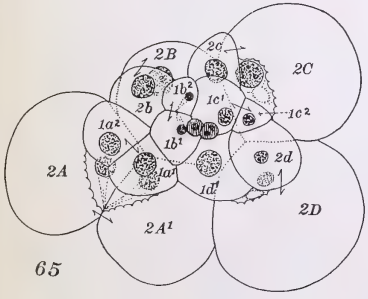

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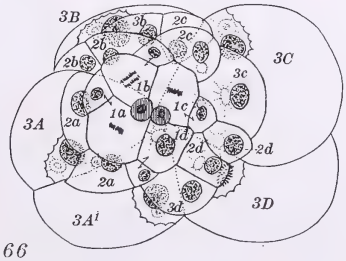

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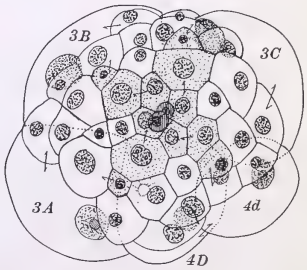

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CONKLIN: NUCLEAR AND CELL DIVISION IN CREPIDULA

EFFECTS OF PRESSURE

PLATE XLVIII.

Effects of Electric Current.

Eggs subjected to electric current (except figs. 76, 77) but probably showing effects of pressure.

Fig. 68. Exp. 1104 (?): 5 mil. amp., 5 min. First maturation spindle; the mitotic figure is much longer than normal, is central in the egg and the spindle fibres, centrosomes and astral rays are either lacking or very faint. The position is probably due to pressure.

Fig. 69. Exp. 1121 (2): 5 mil. amp. 10 min., normal 3½ hrs. Second cleavage spindles abnormal in position, first cleavage furrow incomplete, development stopped.

Fig. 70. Exp. 1121 (2): Similar to preceding.

Fig. 71. Exp. 1121 (2): Similar to preceding.

Fig. 72. Exp. 1121 (2): Similar to preceding.

Fig. 73. Exp. 1121 (2): Two cells, one containing a complete amphiaster but without any chromatin, the other containing two nuclei and one sphere, probably as the result of pressure at the close of the 1st cleavage.

Fig. 74. Exp. 1121 (2): Second cleavage spindle abnormal in position and division delayed in one blastomere.

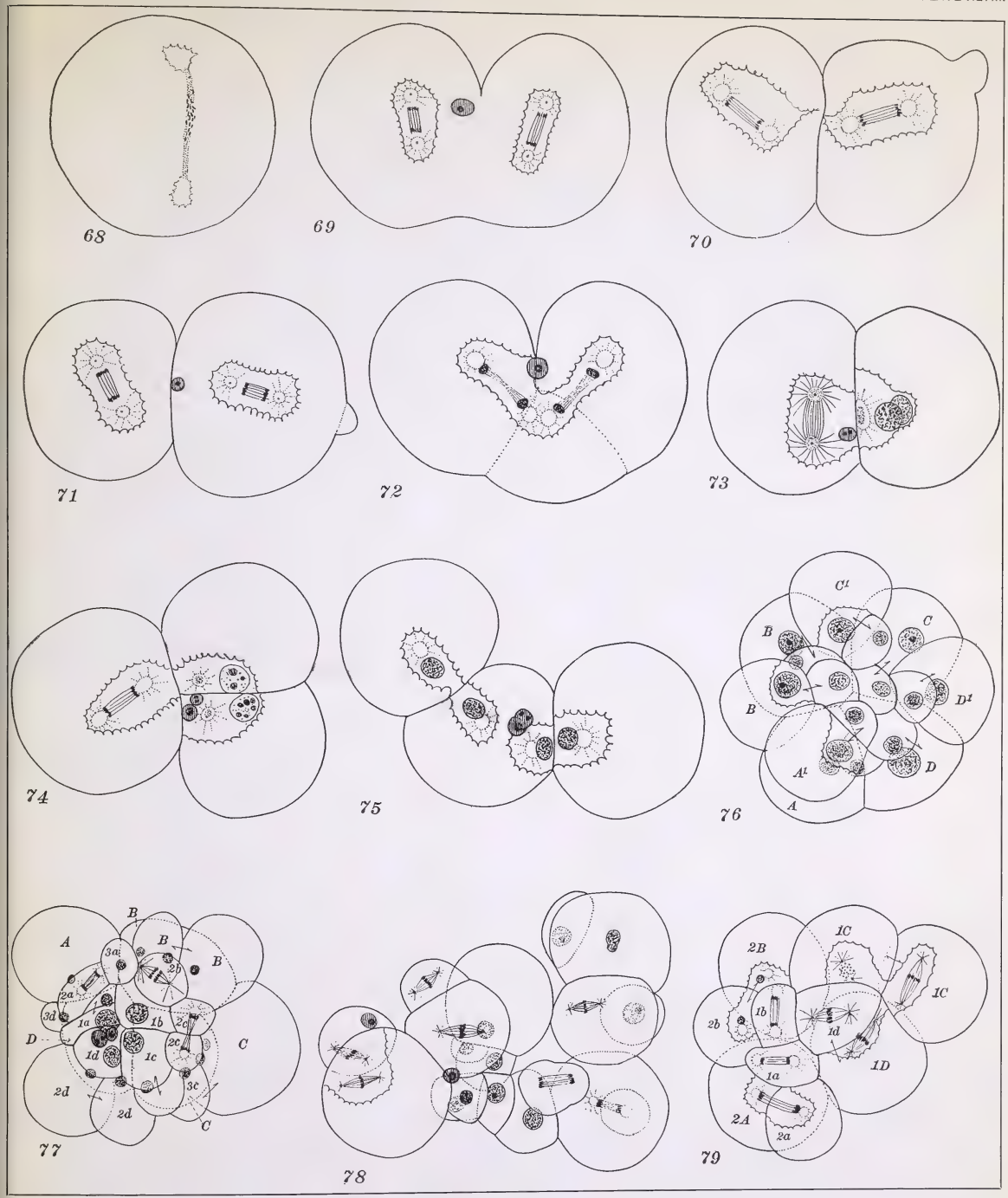
Fig. 75. Exp. 1121 (2): Three macromeres in a linear series, the middle one containing two separate cytoplasmic areas, nuclei and spheres, the result of the suppression of the first cleavage, as in fig. 72.

Fig. 76. Exp. 1001 (3): A pressure experiment included in this plate by mistake, showing eight macromeres and eight micromeres.

Fig. 77. Exp. 919: Egg subjected to pressure, probably after formation of 1st set of micromeres. In quadrant *B* and *D* the 2d set of "micromeres" are really macromeres.

Fig. 78. Exp. 1110 (2): 5 mil. amp., 2 min., normal 22 hrs. Egg with scattered blastomeres in the stage of the formation of the 2d set of micromeres.

Fig. 79. Exp. 1121 (3): 5 mil. amp., 10 min., normal 16 hrs. Egg in stage of formation of 2d set of micromeres, showing effects of pressure.



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CONKLIN: NUCLEAR AND CELL DIVISION IN CREPIDULA

EFFECTS OF ELECTRIC CURRENT

PLATE XLIX.

Effects of Electric Current.

Fig. 80. Exp. 997 (3): 1 volt, 15 min., normal 30 min. Egg and sperm nuclei are large and contain little chromatin, sphere material in granules, no segregation of yolk and cytoplasm.

Fig. 81. Exp. 997 (3): Similar to preceding.

Fig. 82. Exp. 1106: 5 mil. amp., 5 min., normal 17 hrs.; nuclear membrane dissolved and chromatin clumped; development stopped.

Fig. 83. Exp. 1106: Similar to preceding.

Fig. 84. Exp. 1106: Similar to preceding.

Fig. 85. Exp. 997 (3): Similar to fig. 80, but with nuclear membrane gone.

Fig. 86. Exp. 1140 (1): 2 mil. amp., 2 min., normal 5½ hrs. Chromatin disappearing.

Fig. 87. Exp. 1140 (2): 5 mil. amp., 10 min. Ordinary tetraster.

Fig. 88. Exp. 997 (2): 1 volt, 15 min., normal 2½ hrs. Plasma and nuclei displaced by convection current, as in centrifuged eggs.

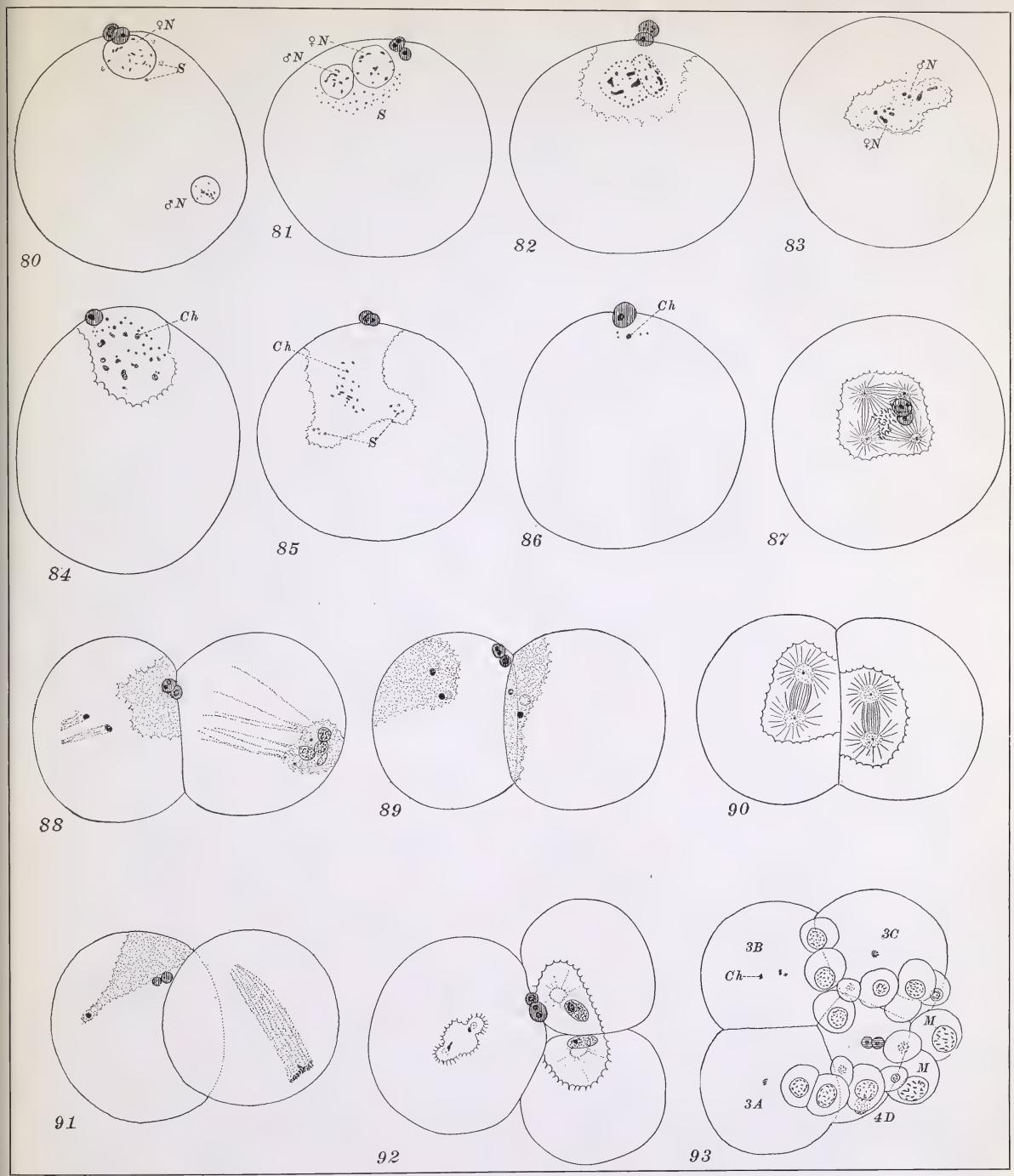
Fig. 89. Exp. 997 (2): Similar to preceding.

Fig. 90. Exp. 1121 (2): Chromosomes have disappeared leaving the spindle fibers a little more chromatic than in normal eggs.

Fig. 91. Exp. 997 (2): 1 volt, 15 min., normal 2½ hrs. Chromatin largely dissolved and displaced toward lower pole; in right cell long strands of cytoplasm.

Fig. 92. Exp. 1121 (2): 5 mil. amp., 10 min., normal 3½ hrs. Spindle and chromosomes disappearing in left cell; others normal.

Fig. 93. Exp. 998 (2): 4 dry cells 1¾ hrs., normal 11 hrs. Evidently egg was in an advanced stage (ca. 42 cells) at the time of the experiment. Although the cells are not dead, the micromeres are rounded (framboisia) and many have dropped off.



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CONKLIN: NUCLEAR AND CELL DIVISION IN CREPIDULA

EFFECTS OF ELECTRIC CURRENT

PLATE L.

Effects of Abnormal Temperature.

Fig. 94. Exp. 1170 (1): Ca. 38° C. 1 hr.; egg irregular in outline, with archiplasm withdrawn into amphiaster, and into the surface layer. First maturation amphiaster irregular in shape and chromosomes scattered; sperm nucleus near vegetal pole.

Fig. 95. Exp. 1174 (2): 37° C. ¼ hr., room temp. (27°) 3 hrs.; first polar body very large; chromosomes of second maturation division have formed karyomeres; sperm nucleus near animal pole.

Fig. 96. Exp. 1171 (1): Ca. 35° C. ½ hr.; 2-cell stage, showing dense aggregation of archiplasm around nuclei and spheres, with different kinds of cytoplasm in other parts of cell.

Fig. 97. Exp. 1171 (1): Similar to preceding; second cleavage spindles greatly modified; chromosomes scattered; archiplasm gathered in spindle areas and division wall.

Fig. 98. Exp. 1171 (2): Ca. 35° C. ½ hr., room temp. (ca. 24°–26°) 15 hrs.; the archiplasm has collected in central areas in each cell and in division walls; chromosomes are clumped and thrown out of cytoplasmic areas.

Fig. 99. Exp. 1171 (2): Similar to preceding.

Fig. 100. Exp. 962: On ice 16 hrs.; the spheres of the third cleavage are unusually distinct and the scattered sphere granules of the second cleavage (in the micromeres) are very large.

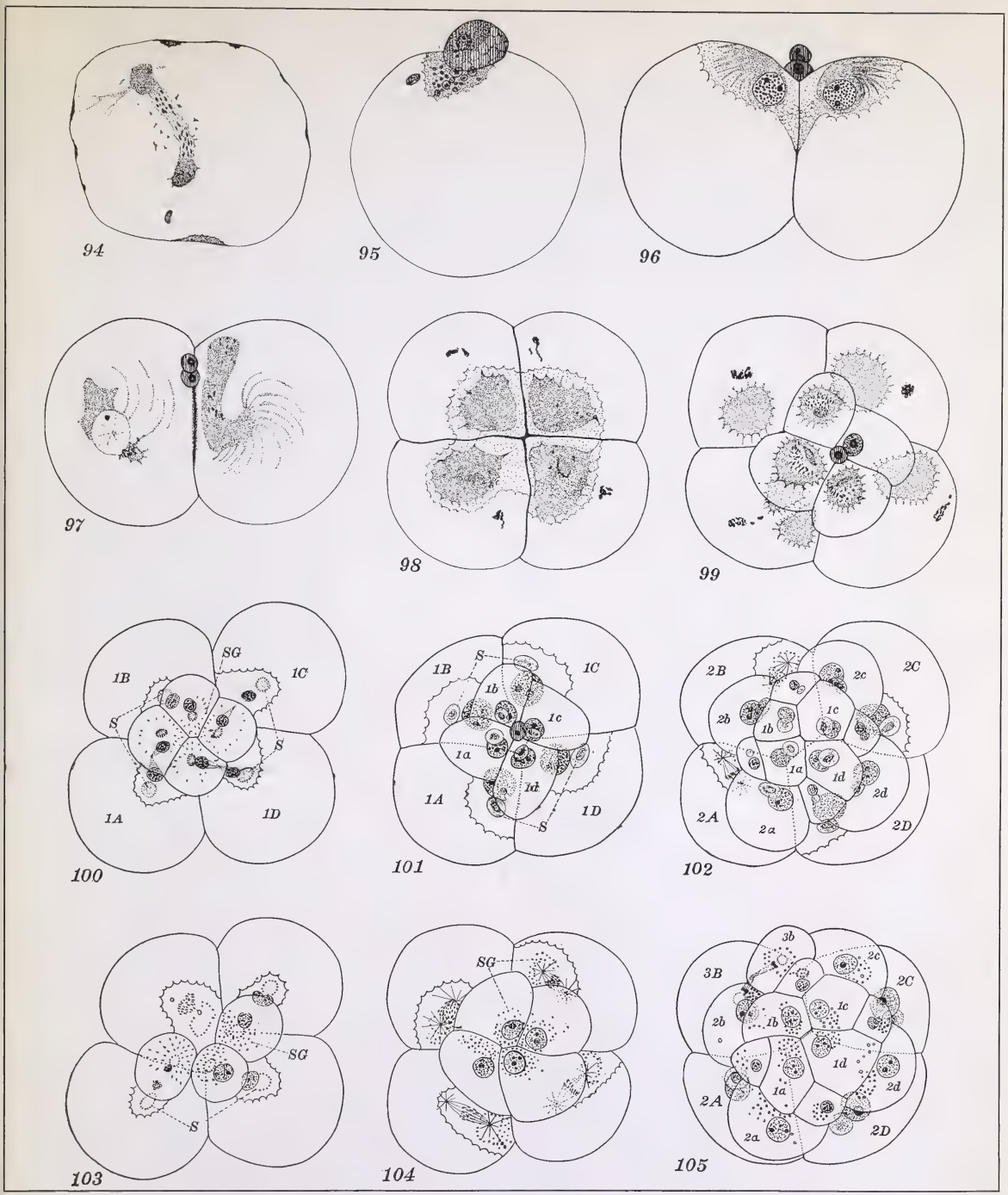
Fig. 101. Exp. 962: The spheres have a definite boundary, stain more deeply than usual and look almost like nuclei.

Fig. 102. Exp. 962: A later stage, with spheres similar to those shown in fig. 101.

Fig. 103. Exp. 964: On ice 40 hrs.; the sphere granules are especially large.

Fig. 104. Exp. 964: Similar to preceding.

Fig. 105. Exp. 964: Similar to preceding; many of the sphere granules are vesicular.



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CONKLIN: NUCLEAR AND CELL DIVISION IN CREPIDULA

EFFECTS OF ABNORMAL TEMPERATURES

PLATE LI.

Effects of Decreased Oxygen Tension.

Figs. 106–109. Exp. 1010: Eggs placed for 36 hrs. in sea water which had been boiled to drive off contained gases, and then cooled; all development was completely stopped, but eggs were not killed; chromatin in the resting nuclei is collected into one or more masses; spindle fibres are distinct but astral rays are lacking; centrosomes and sphere granules are vesicular. The eggs used in this experiment were much smaller than normal, being only 120 μ in diameter.

Figs. 110–111. Exp. 1025: Eggs left for 18 hrs. in a stoppered bottle of sea water, through which hydrogen had been run for 1 hr.; development completely stopped; nuclei and nucleoli large, little chromatin.

Fig. 112. Exp. 1016: Eggs placed for 27 hrs. in sea water which had been boiled and cooled; similar to preceding, development stopped.

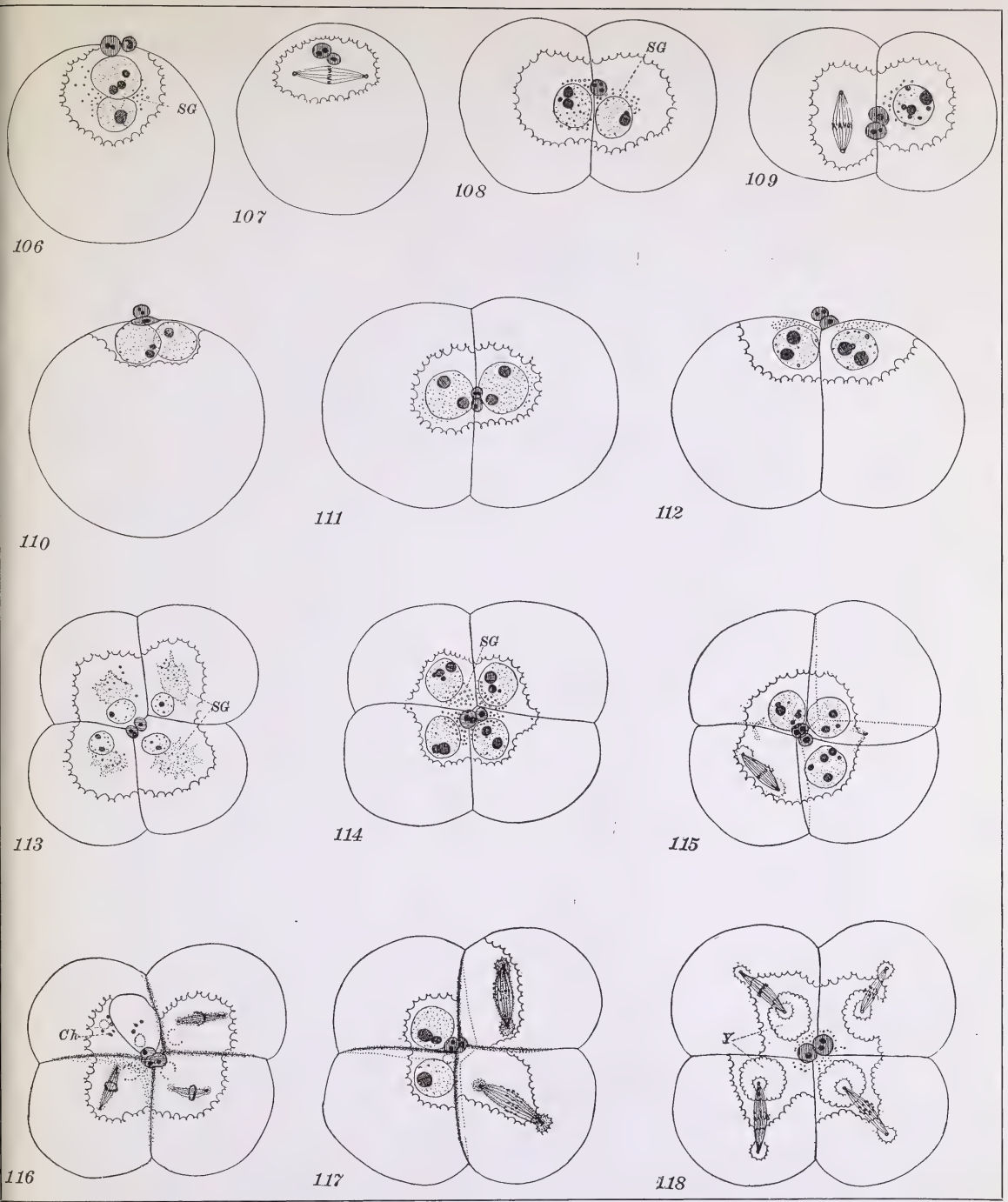
Fig. 113. Exp. 1017: Eggs left for 48 hrs. in stoppered test tube of boiled and cooled sea water; development completely stopped; sphere granules prominent.

Fig. 114. Exp. 1016: Same as fig. 112; nuclei with little chromatin; sphere granules prominent.

Fig. 115. Exp. 1023: Eggs subjected to atmosphere of hydrogen for 2 hrs. and then left in open bottle for 2 hrs.; development stopped; eggs similar to all others subjected to decreased oxygen tension.

Fig. 116. Exp. 1017: Same as in fig. 113: The spindles are small, deep-staining and without astral rays; the chromosomes are arranged in a ring around the spindles.

Figs. 117–118. Exp. 1025: Eggs left for 18 hrs. in stoppered bottle of sea water through which hydrogen had been run for 1 hr.; eggs similar to fig. 115; in fig. 118 there is an area of yolk (*Y*) around the upper poles of the spindles.



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CONKLIN: NUCLEAR AND CELL DIVISION IN CREPIDULA

DECREASED OXYGEN TENSION

PLATE LII.

Effects of Carbonic Acid.

Eggs in 1–8-cell stage left for 28 hrs. in sea water ½ saturated with CO_2.

Fig. 119. Exp. 1163: Egg showing the individually distinct chromosomal vesicles of the maturation divisions; also the cell membrane separated from the subjacent egg substance.

Fig. 120. Exp. 1163: Eggs showing five protoplasmic cells (one of them with four nuclei) on the unsegmented yolk.

Fig. 121. Exp. 1164: Side view of an egg similar to the preceding.

Fig. 122. Exp. 1163: The second polar body is abnormally large; an accessory aster (*S*) lies in the yolk; the division of the nucleus in the 1st cleavage has taken place normally, though the spheres are prevented from moving to their normal positions above the nuclei by the presence of the large polar body; the cleavage furrow cuts into the egg from the animal pole side only, and ends in a "cleavage head" as in cœlenterate eggs.

Fig. 123. Exp. 1163: The *C* and *D* quadrants are entirely normal; in the blastomere *AB*, the nucleus divided but the cell did not; these two nuclei in an undivided cell gave off a single blastomere of the first set (1*ab*) with large lobulated nucleus, the nuclei, spheres and cytoplasmic areas of the cell *AB* then came to lie at opposite sides of the macromere and each divided independently giving rise to a second micromere (2*a*, 2*b*) which is nearly normal.

Fig. 124. Exp. 1163: There are three macromeres one of which contains a perfect spindle but no chromatin; another contains a polyaster and has given off a large micromere with three nuclei; the third contains a single nucleus which is smaller than normal and has given off in reversed cleavage a 1st and a 2d micromere, the former of which is dividing.

Fig. 125. Exp. 1163: The first cleavage furrow failed to appear though the nuclei divided; at the 2d cleavage each nucleus with its adjacent oöplasm gave off a smaller macromere (*B* and *D*), leaving macromeres *A* and *C* still undivided; each of these four macromeres has given off a micromere of the 1st set.

Fig. 126. Exp. 1163: Three macromeres one of which contains several centrosomes and spheres (*S*) but no nucleus; another contains a bifurcated spindle and a normal one and has just given off two micromeres one on the right and one on the left; the other macromere contains a single nucleus and sphere and has just given off a micromere on the right; two large micromeres with abnormal nuclei occupy the center of the micromere field. There is a general resemblance of this egg to that shown in fig. 124.

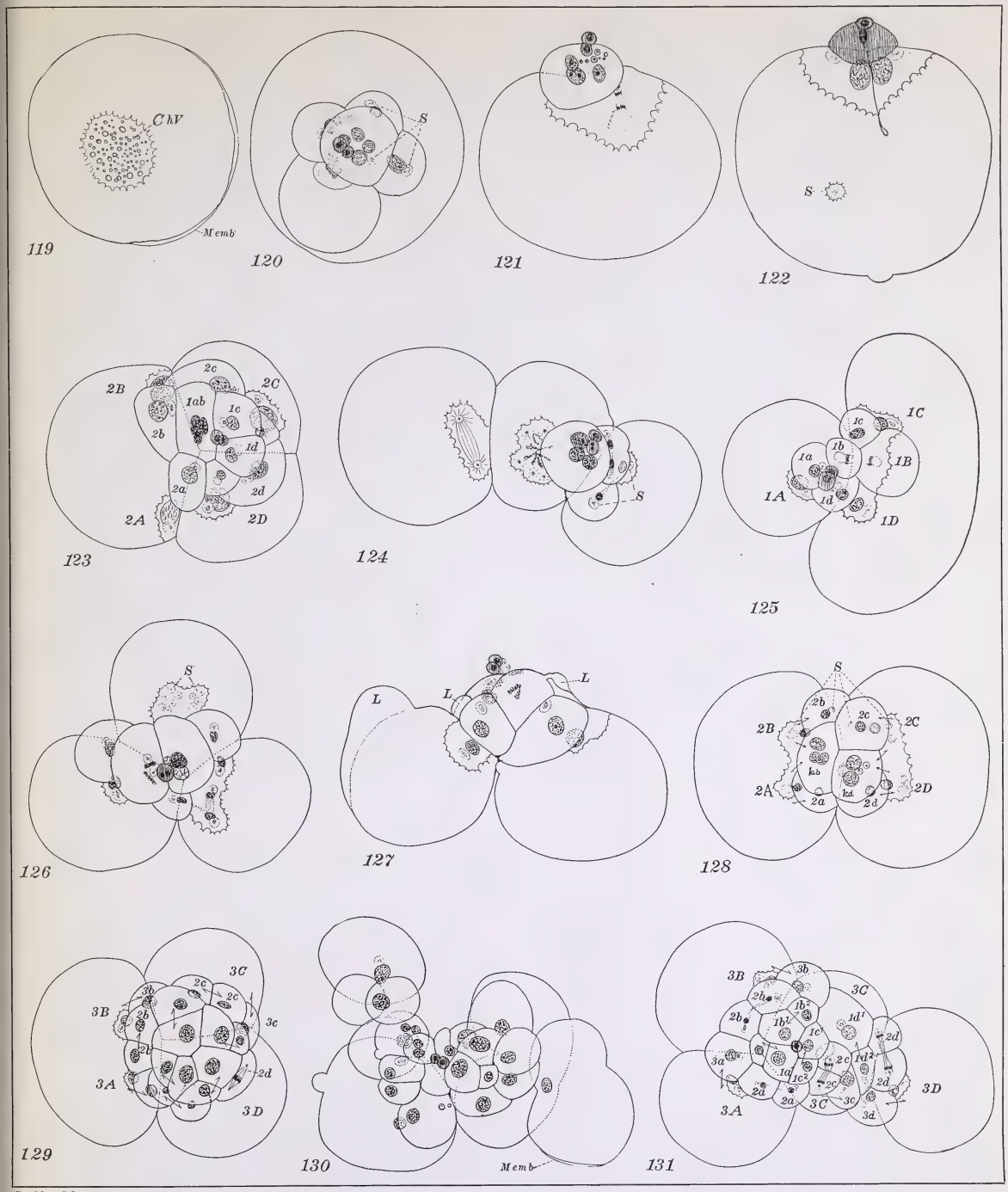
Fig. 127. Exp. 1163: Side view of egg with two macromeres and several micromeres. Strands of protoplasm run from the polar bodies to the micromeres and from one of the latter to a macromere, suggesting the "spinning" activities of other eggs; lobes are also found on several cells.

Fig. 128. Exp. 1163: Two macromeres, each with two nuclei and two or more spheres; in the second cleavage the nuclei divided but the cell-body did not; in the third cleavage there was probably a triaster in each macromere, since only two micromeres of the first set were formed (1*ab*, 1*cd*) each with multiple nuclei; in the fourth cleavage each macromere contained two separate spindles and gave off two separate micromeres (2*a*, 2*b*, 2*c*, 2*d*) of the second set; the nuclei in the macromeres are so well separated that it is probable that at the next cleavage two independent spindles would form in each macromere and would lead to the formation of four micromeres of the third set.

Fig. 129. Exp. 1163: Macromeres *A* and *B* did not separate at the 2d cleavage, but each has given rise to three micromeres forming a typical micromere plate, though the direction of division in *A* and *B* has sometimes been atypical.

Fig. 130. Exp. 1164: Irregular cleavage mass in which it is not possible to identify many cells. Several of the cells show loose membranes and lobes.

Fig. 131. Exp. 1163: One of the macromeres (*D*) was separated from the other three, but each has given rise to three micromeres which have subdivided in normal manner, the micromeres formed from *D* lying on the right of the micromere plate formed from the other macromeres.



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CONKLIN: NUCLEAR AND CELL DIVISION IN CREPIDULA

EFFECTS OF CARBONIC ACID

PLATE LIII.

Effects of Diluted Sea Water.

In figs. 132–135, 137–140, 144 the dilution was one part sea water to two parts fresh water; in all other cases the sea water was diluted with equal parts of fresh water. With higher dilutions the blastomeres tend to separate but do not swell appreciably.

Fig. 132. Exp. 875: Second polar spindle at animal pole; sperm nucleus has formed a spindle (♂*Sp*); the homogeneous chromatic sphere below this may represent an accessory sperm nucleus (♂*N*).

Fig. 133. Exp. 875: First cleavage spindle; the seven chromatic spheres may represent accessory sperm nuclei (♂*N*).

Fig. 134. Exp. 875: Enormous second polar body containing large nucleus and yolk; two nuclei and accessory sperm nucleus (♂*N*) in egg.

Fig. 135. Exp. 875: Probably ½ blastomere containing polyaster and with a micromere which has just divided.

Fig. 136. Exp. 872: Three blastomeres showing reversed polarity, the spheres, nuclei and cytoplasmic areas lying at the pole opposite the polar bodies; one sphere is found in each cell but in the two larger ones the nuclei are multiple.

Fig. 137. Exp. 875: Two macromeres, one containing a triaster, the other a tetraster; the two micromeres are normal except for their large size.

Fig. 138. Exp. 875: Similar to the preceding.

Fig. 139. Exp. 875: Side view of an egg similar to figs. 137, 138.

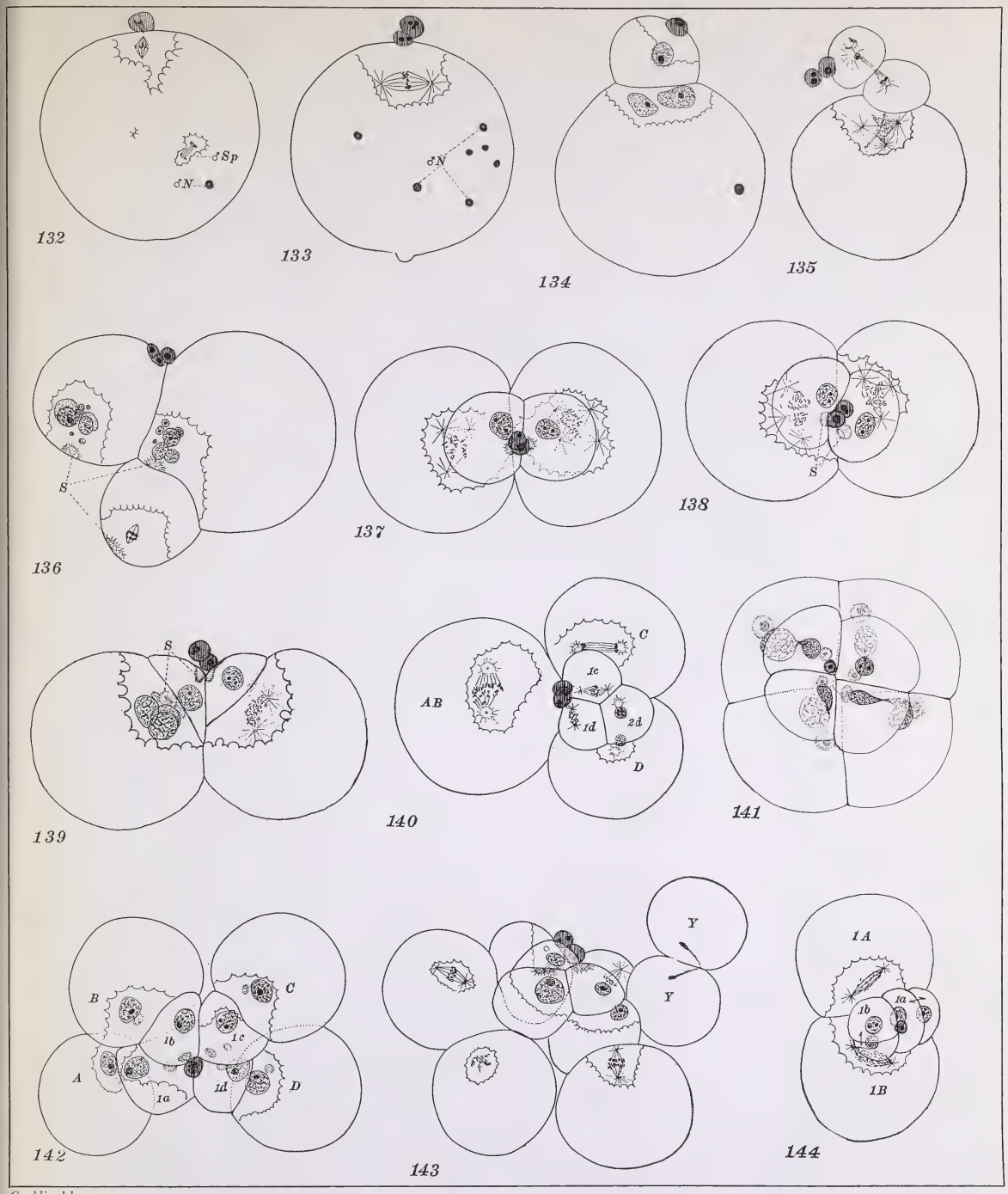
Fig. 140. Exp. 875: Macromeres *A* and *B* have not divided and the chromosomes are irregularly scattered in the spindle; macromeres *C* and *D* have divided normally giving rise to first and second micromeres and the first set are subdividing normally.

Fig. 141. Exp. 859: Chromosomes were scattered along the spindle during the third cleavage and have given rise to chromatic connections between daughter nuclei, which resemble amitoses.

Fig. 142. Exp. 872: The micromeres are larger than usual (two of them contain yolk) and they have caused a separation of *A*, *B*, from *C*, *D*.

Fig. 143. Exp. 872: The micromeres are larger than usual and contain yolk; the macromeres are separated and one which has just divided (*Y*, *Y*) contains yolk but no cytoplasmic areas; the chromosomes are here scattered along the spindle axis, thus forming a chromatic connection.

Fig. 144. Exp. 875: ¾ blastomeres, each of which has given rise to one micromere, which has subdivided; the macromeres contain spindles along which the chromosomes are scattered irregularly.



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CONKLIN: NUCLEAR AND CELL DIVISION IN CREPIDULA

DILUTED SEA WATER

PLATE LIV.

Effects of Diluted Sea Water.

In all experiments represented on this plate sea water was diluted with equal parts of distilled water

Fig. 145. Exp. 858: Several micromeres have been formed but the yolk has not divided; three of the cells contain several nuclei and spheres, the result probably of polyasters, and one contains a tetraster.

Fig. 146. Exp. 993 (1): Similar to the preceding; the protoplasmic micromeres are partly constricted from the yolk.

Fig. 147. Exp. 858: Exogastrula; similar to the preceding but of a more advanced stage; the multinucleate yolk cell is uncovered by the ectoderm.

Fig. 148. Exp. 993: Similar to the preceding.

Fig. 149. Exp. 993: Side view of an egg placed in diluted sea water in the 2-cell stage; the second cleavage of the yolk was suppressed, but several micromeres have been formed from each macromere.

Fig. 150. Exp. 956: Egg similar to the preceding, viewed from the animal pole; each macromere contains a large quadripartite nucleus and has given rise to twelve micromeres, which cannot be individually identified.

Fig. 151. Exp. 993: Isolated $\frac{1}{2}$ blastomere, the yolk cell has not divided, but contains several nuclei and has given rise to nine micromeres.

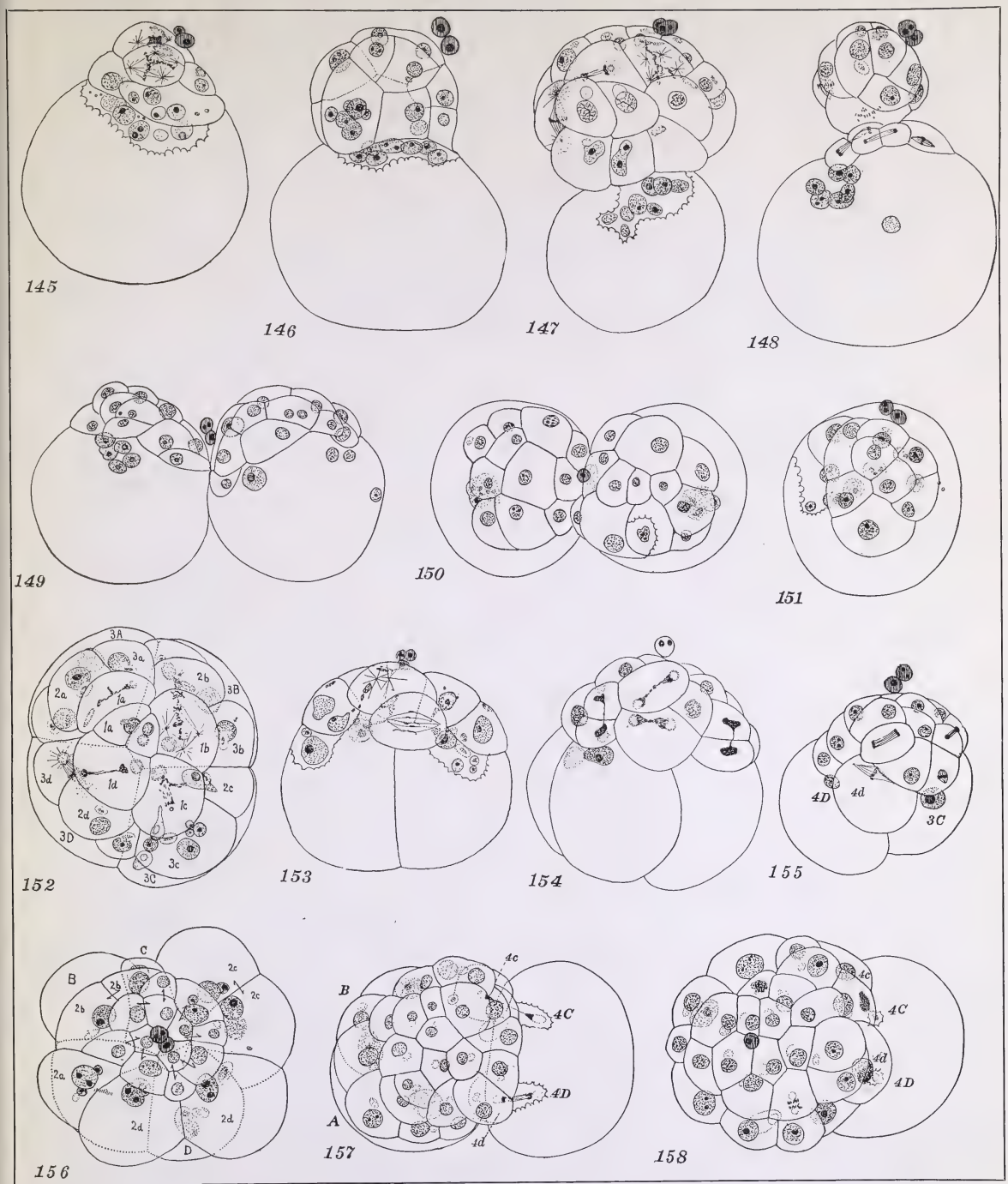
Figs. 152, 153. Exp. 858: Top and side views of eggs which were placed in diluted sea water after formation of the 1st set of micromeres; several dividing cells contain triasters or tetrasters and the chromosomes are widely scattered; chromatic connections between daughter nuclei are falsely suggestive of amitosis.

Fig. 154. Exp. 858: Side view of egg placed in diluted sea water after formation of the three sets of micromeres which are approximately normal; scattered chromosomes have given rise to chromatic connections between daughter nuclei.

Fig. 155. Exp. 993: Isolated $\frac{2}{4}$ blastomeres which have produced a $\frac{2}{4}$ micromere plate; the macromere 4*D* has given off the mesentoblast 4*d* which is now dividing in normal manner.

Fig. 156. Exp. 871: The 1st set of micromeres have divided twice in normal directions, as indicated by the arrows, giving rise to twelve micromeres; in the formation of the 2d set of micromeres the division of the macromeres was approximately equal.

Figs. 157, 158. Exp. 858: Eggs in which the nuclear division at the 2d cleavage took place normally, but in which the cell body did not divide; three quartets of micromeres were formed and have subdivided in approximately normal manner, although there are only two separate macromeres. The 4th quartet cells 4*d* and 4*c* form simultaneously from the undivided macromere *CD*, though in normal eggs 4*d* (the mesentoblast) forms at the 24-cell stage, while 4*c* (an entoblast) does not form until the 52-cell stage.



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CONKLIN: NUCLEAR AND CELL DIVISION IN CREPIDULA

DILUTED SEA WATER

PLATE LV.

Effects of Hypertonic Sea Water.

All eggs shown on this plate are from Exp. 804, and were subjected to 1 per cent. NaCl in sea water for 4 hrs.

Figs. 159–163. The sperm nucleus lies in a small area of cytoplasm near the lower pole; the egg nucleus lies in a larger area of cytoplasm at the animal pole; various stages in the formation of the egg spindle are shown.

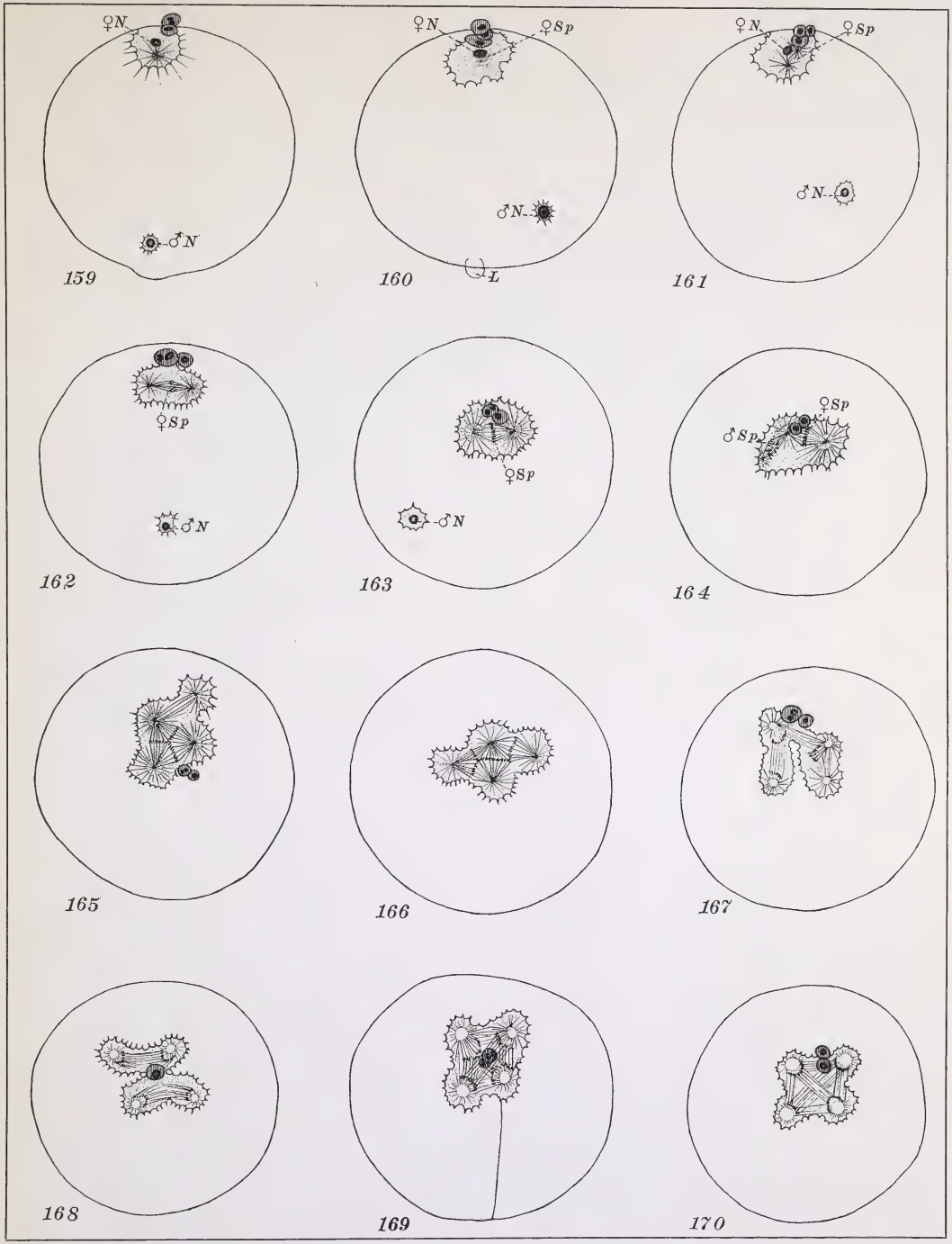
Fig. 164. Two spindles, probably those of egg and sperm, are joined at one pole.

Figs. 165, 166. Tetrasters, probably formed from the egg and the sperm spindles.

Fig. 167. Two spindles, probably those of the egg and the sperm, joined at one pole.

Fig. 168. Two spindles, probably those of the egg and sperm, quite separate.

Figs. 169, 170. Tetrasters in different phases of the separation of the chromosomes.



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CONKLIN: NUCLEAR AND CELL DIVISION IN CREPIDULA

HYPERTONIC SEA WATER

PLATE LVI.

Effects of Hypertonic Sea Water.

Fig. 171. Exp. 823: 3 per cent. NaCl, 16 hrs., normal 8 hrs. Egg nucleus very small and clear with chromosomes persistent within vesicle and egg centrosome outside vesicle; the sperm nucleus is enormous and contains much chromatic sap.

Fig. 172. Exp. 823: Similar to the preceding.

Fig. 173. Exp. 837: 1 per cent. KCl, 9 hrs., normal 35 hrs. Development has been stopped but the egg is not dead; the germ nuclei are very large and achromatic; small achromatic spherules lie in the cytoplasm.

Fig. 174. Exp. 822: 2 per cent. NaCl, 16 hrs., normal 8 hrs. Achromatin in large and small vesicles in the cytoplasm; germ nuclei normal.

Fig. 175. Exp. 822: The germ nuclei are broken up into many separate vesicles, each with a chromatic nucleolus.

Fig. 176. Exp. 823: Two-cell stage from same experiment as figs. 171, 172; nuclei very large and achromatic.

Fig. 177. Exp. 822: Side view of egg in 2-cell stage, each cell containing a large sphere (*S*) and one or more chromatic and two or three achromatic nuclear vesicles.

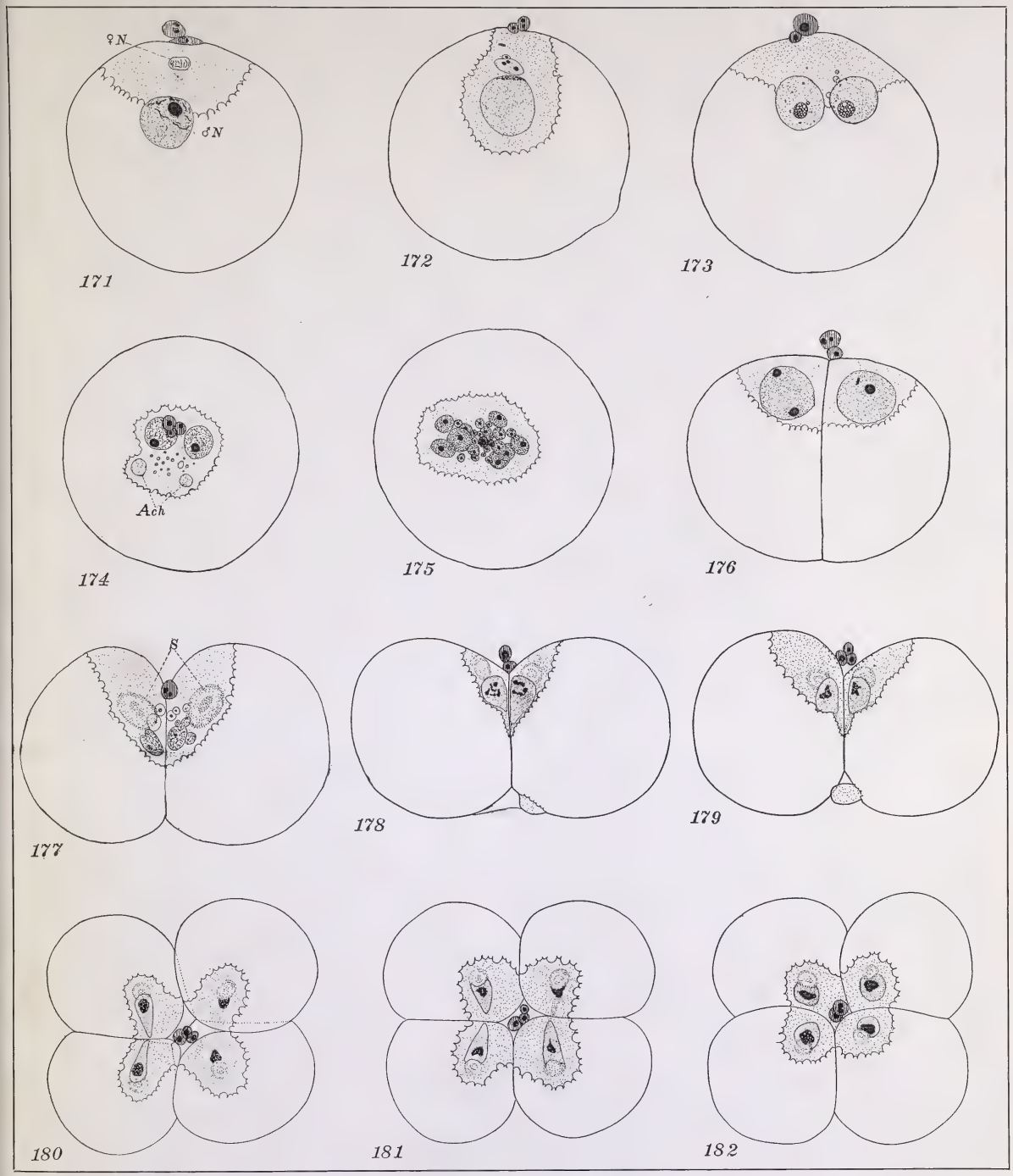
Fig. 178, 179. Exp. 810: 3 per cent. NaCl, 15 hrs.: Side views of egg in 2-cell stage showing the chromatin massed within the nuclear vesicle; the latter are elongated along the line of the former spindle axis.

Figs. 180–182. Same experiment as preceding; 4-cell stages from animal pole.

Fig. 180. In one half of egg the remains of the 2d cleavage spindle are still visible and the chromatin has formed no nuclear vesicle; in the other half the nuclear vesicles are elongated along the line of the spindle axis, the chromatin being massed at the ends of the vesicle nearest the spheres.

Fig. 181. Nuclear vesicles elongated along the spindle axis are present in three of the cells, and in each the chromatin is massed at the end of the vesicle nearest the sphere; in the fourth cell no nuclear vesicle is present, but traces of spindle fibres may be seen.

Fig. 182. In all four cells the nuclear vesicles are rounded and the chromatin is massed near the center of each vesicle.



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CONKLIN: NUCLEAR AND CELL DIVISION IN CREPIDULA

HYPERTONIC SEA WATER

PLATE II.

[illegible]

Figs. 183–188 [illegible]

Fig. 184. [illegible]

Figs. 185, 186. [illegible]

Fig. 188. [illegible]

Fig. 189. [illegible]

Fig. 190. [illegible]

Fig. 191. [illegible]

Figs. 192, 193. [illegible]

Figs. 194, 195. [illegible]

Figs. 196, 197. [illegible]

Figs. 198, 199. [illegible]

PLATE LVII.

Effects of Hypertonic Sea Water.

Figs. 183–185 were in 1-cell stage at beginning of experiment; Figs. 186–196 were in 2-cell stage.

Fig. 183. Exp. 805: 2 per cent. NaCl, 4 hrs.; in both egg and sperm nuclei the chromatin is aggregated into a dense mass in the center of the nuclear vesicle; there are many cytasters near the sperm nucleus.

Fig. 184. Exp. 809: 2 per cent. NaCl, 15 hrs.; the chromatic and achromatic parts of the germ nuclei are in separate vesicles.

Fig. 185. Exp. 809: Similar to the preceding; a double cytaster is present near the germ nucleus.

Fig. 186. Exp. 809: 2 per cent. NaCl, 15 hrs.; the chromatic and achromatic parts of the nucleus are in separate vesicles; the achromatic vesicles are numerous and scattered.

Fig. 187. Exp. 809: Side view of egg similar to preceding.

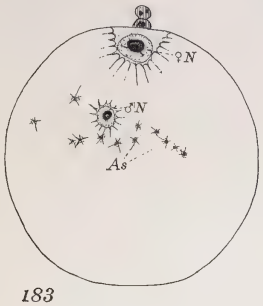
Fig. 188. Exp. 809: Egg similar to the preceding showing two principal achromatic vesicles (*Ach*) and many smaller vesicles or sphere granules.

Figs. 189, 190. Exp. 805: 2 per cent. NaCl, 4 hrs.; the 2d cleavage spindles are much shrunken in size; the archiplasm around them is much condensed while many small radiating masses of archiplasm are left along the astral radiations as cytasters.

Figs. 191, 192. Exp. 823: 3 per cent. NaCl, 16 hrs., normal 8 hrs.; top and side views of eggs showing in addition to the principal masses of chromatin (*Ch*) very numerous granules or spherules which probably represent scattered achromatic material (*Ach*).

Figs. 193, 194. Exp. 837: 1 per cent. KCl, 9 hrs., normal 36 hrs.; the numerous karyomeres have begun to absorb achromatin and to fuse together.

Figs. 195, 196. Exp. 803: 1 per cent. NaCl, 15 hrs.; top and side views of eggs with two macromeres and two micromeres, each with many masses of chromatin (karyomeres) and several spheres or asters.



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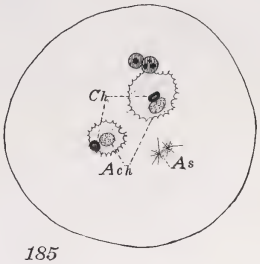

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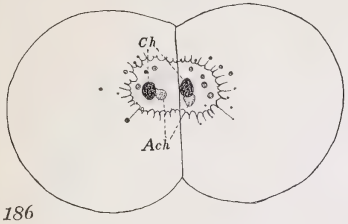

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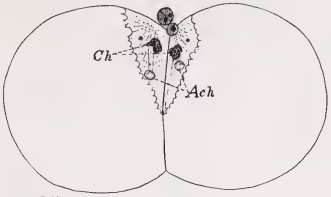

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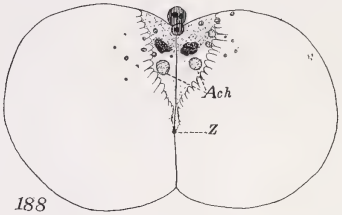

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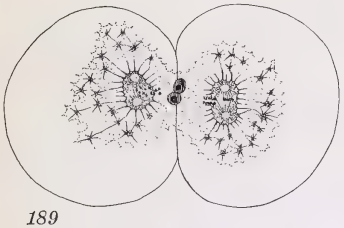

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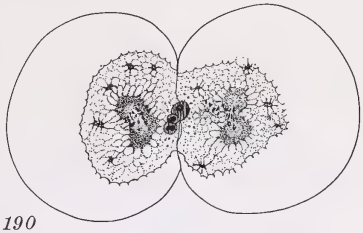

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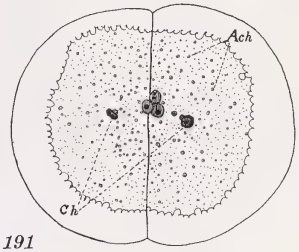

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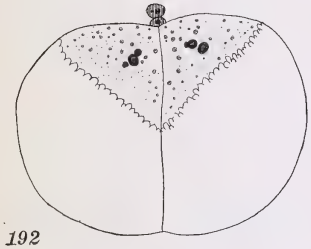

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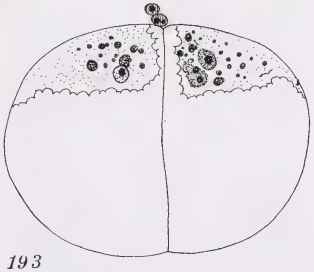

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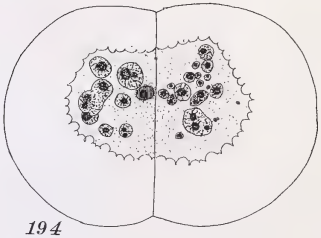

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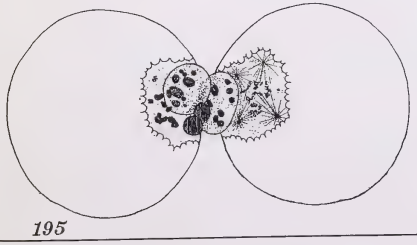

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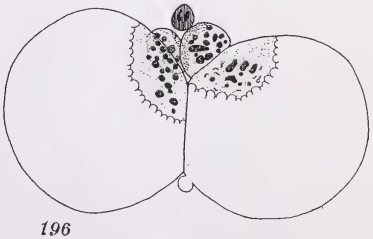

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CONKLIN: NUCLEAR AND CELL DIVISION IN CREPIDULA

HYPERTONIC SEA WATER

PLATE LVIII.

Effects of Hypertonic Sea Water.

All eggs represented on this plate were put into hypertonic solutions in the 1-cell stage, and with the exception of fig. 197 none of them show division of the yolk.

Fig. 197. Exp. 842: 4 per cent. $MgCl_2$, 5 hrs., normal 17 hrs.; the two cells at the upper pole are probably enormous polar bodies, both of which contain mitotic figures.

Fig. 198. Exp. 991: 1 per cent. $MgCl_2$, 18 hrs.; one large cell (macromere) contains all the yolk, two smaller cells (micromeres) are purely protoplasmic; both macromeres and micromeres contain many nuclei (karyomeres), the chromatin of each being condensed into one or two masses.

Fig. 199. Exp. 834: 3 per cent. $MgCl_2$, 9 hrs., normal 9 hrs.; both macromere and micromere contain mitotic figures with many poles and scattered chromosomes; sphere granules at the animal pole of the micromere.

Fig. 200. Exp. 833: 2 per cent. $MgCl_2$, 9 hrs., normal 9 hrs.; resting stage following a division stage like that of the preceding figure.

Fig. 201. Exp. 838: Herbst's Ca-free sea water, 18 hrs.; micromere and macromere containing many nuclei and cytasters.

Fig. 202. Exp. 825: 2 per cent. NaCl, 16 hrs., normal 23 hrs.; two spheres and many scattered nuclei (karyomeres) in both macromere and micromere.

Fig. 203. Exp. 827: $\frac{3}{4}$ per cent. NaCl, 9 hrs., normal 8 hrs.; many asters and scattered chromosomes in the macromere and in one of the micromeres.

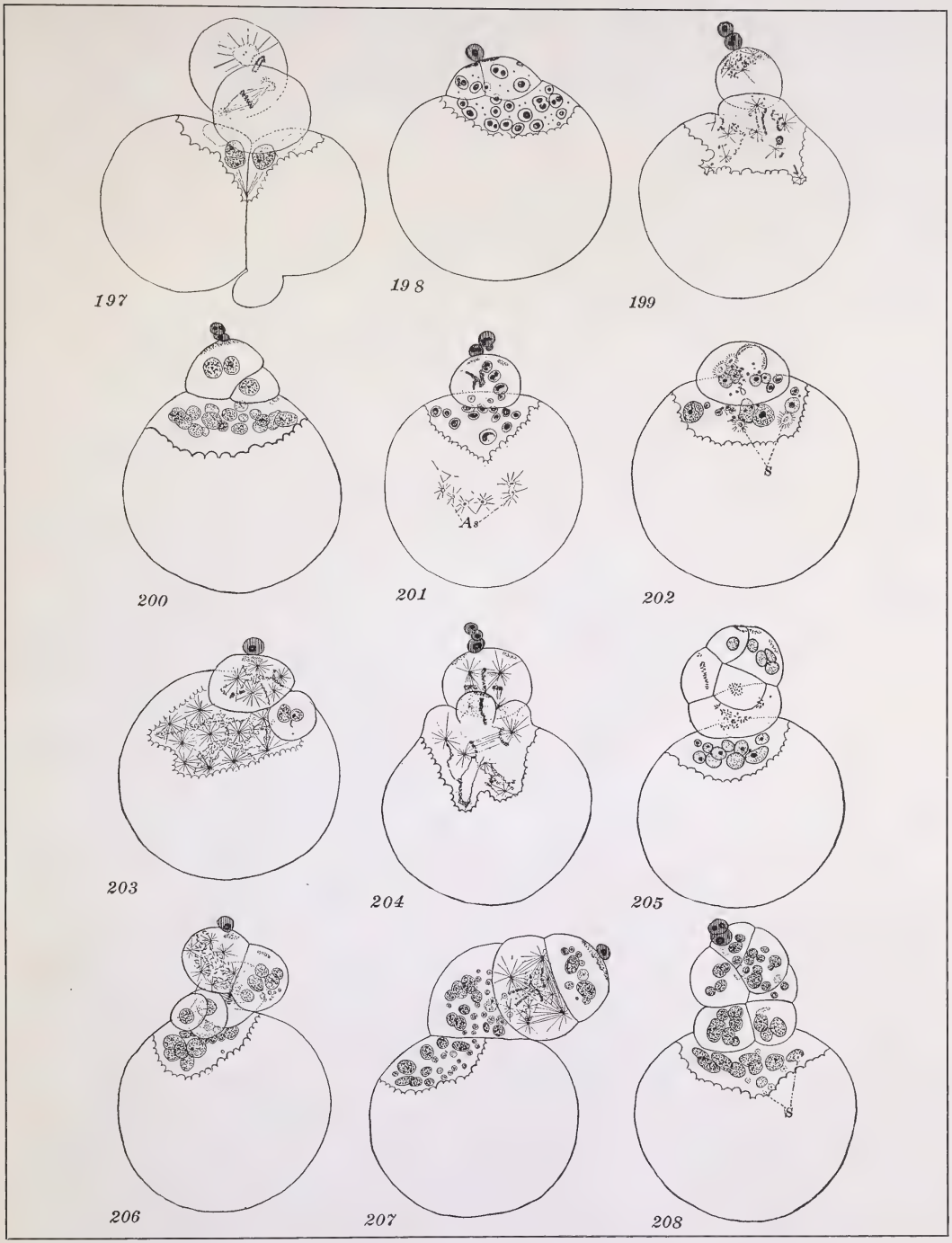
Fig. 204. Exp. 863: 2 per cent. NaCl, $2\frac{1}{2}$ hrs., normal 10 hrs.; similar to the preceding; scattered spindles and chromosomes; three small micromeres (lobes) budding off from the macromere; sphere granules at the animal pole of the micromeres.

Fig. 205. Exp. 843: 1 per cent. NaCl, 6 hrs., normal 15 hrs.; several micromeres, three of them containing several nuclei or groups of chromosomes; the macromere contains many nuclei (karyomeres).

Fig. 206. Exp. 827: $\frac{3}{4}$ per cent. NaCl, 9 hrs., normal 8 hrs.; macromere and micromeres containing many karyomeres and asters.

Fig. 207. Exp. 830: 2 per cent. NaCl, 9 hrs., normal 32 hrs.; macromeres and micromeres containing a large number of karyomeres or asters; sphere granules under the polar body.

Fig. 208. Exp. 833: 2 per cent. $MgCl_2$, 9 hrs., normal 9 hrs.; many nuclei (karyomeres) and spheres in each cell.



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CONKLIN: NUCLEAR AND CELL DIVISION IN CREPIDULA

HYPERTONIC SEA WATER

PLATE LIX.

Effects of Hypertonic Sea Water.

Eggs represented in figs. 209–218 were in the 2-cell stage when the experiments began, and in none of them did the yolk undergo any subsequent cleavage; figs. 219–223 were in the 4-cell stage at the beginning of the experiment.

Fig. 209. Exp. 865: 4 per cent. NaCl, 2 hrs., normal 10 hrs.; the nuclear division at the 2d cleavage occurred regularly but the cell division was suppressed, thus leaving two nuclei of normal appearance, in each cell; at least three spheres are present in the left cell.

Fig. 210. Exp. 804: 1 per cent. NaCl, 4 hrs.; partial suppression of the 2d cleavage furrow which is limited to a shallow furrow on the animal pole side of the egg; the nuclei are irregular and densely chromatic.

Fig. 211. Exp. 864: 3 per cent. NaCl, 3 hrs., normal 10 hrs.; the 2d cleavage spindles have the position of the 3d cleavage spindles of normal eggs; the chromosomes are scattered abnormally on the spindles.

Fig. 212. Exp. 865: 4 per cent. NaCl, 2 hrs., normal 10 hrs.; the two macromeres have each given off a first and a second micromere, the first by dexiotropic and the second by læotropic division, as in normal eggs. Each of the first micromeres contain a single nucleus and sphere; the second micromeres and the macromeres contain several spheres and karyomeres or groups of chromosomes.

Fig. 213. Exp. 863: 2 per cent. NaCl, 2½ hrs., normal 10 hrs.; the first set of micromeres have been formed in dexiotropic direction, as in normal cleavage; the macromeres are dividing again with double spindles or tetrasters.

Fig. 214. Exp. 846: 4 per cent. $MgCl_2$, 4 hrs., normal 16 hrs.; the two macromeres are separated by a group of micromeres which have formed along both sides of the 1st cleavage plane; the polarity of each half thus appears to have been changed, the centers of the ectodermal pole being the surface of contact between the two halves; this may be due, in part, to the outward rotation of the macromeres at the vegetal pole and their inward rotation at the animal pole.

Fig. 215. Exp. 846: 4 per cent. $MgCl_2$, 4 hrs., normal 16 hrs.; side view of an egg with two macromeres and six micromeres; the two original micromeres of the first set have subdivided as indicated by the arrows, the other two micromeres are of the 2d set; all the micromeres contain karyomeres; the macromeres contain chromosomes scattered apparently, along the line of the 1st cleavage spindle.

Fig. 216. Exp. 846: Egg from same experiment as the preceding; the nuclei at the vegetal pole are probably derived from chromosomes which were scattered along the first cleavage spindle as in fig. 215.

Fig. 217. Exp. 822: 2 per cent. NaCl, 16 hrs., normal 8 hrs.; irregular and unequal 2d cleavage with several karyomeres of varying size in each cell.

Fig. 218. Exp. 842: 4 per cent. $MgCl_2$, 5 hrs., normal 17 hrs.; the divisions of the cell body at the 2d cleavage were suppressed in the left half, but two micromeres are arising in normal manner from this half; several accessory asters are present in this half which have served to scatter the chromosomes. The right half of the egg is quite normal.

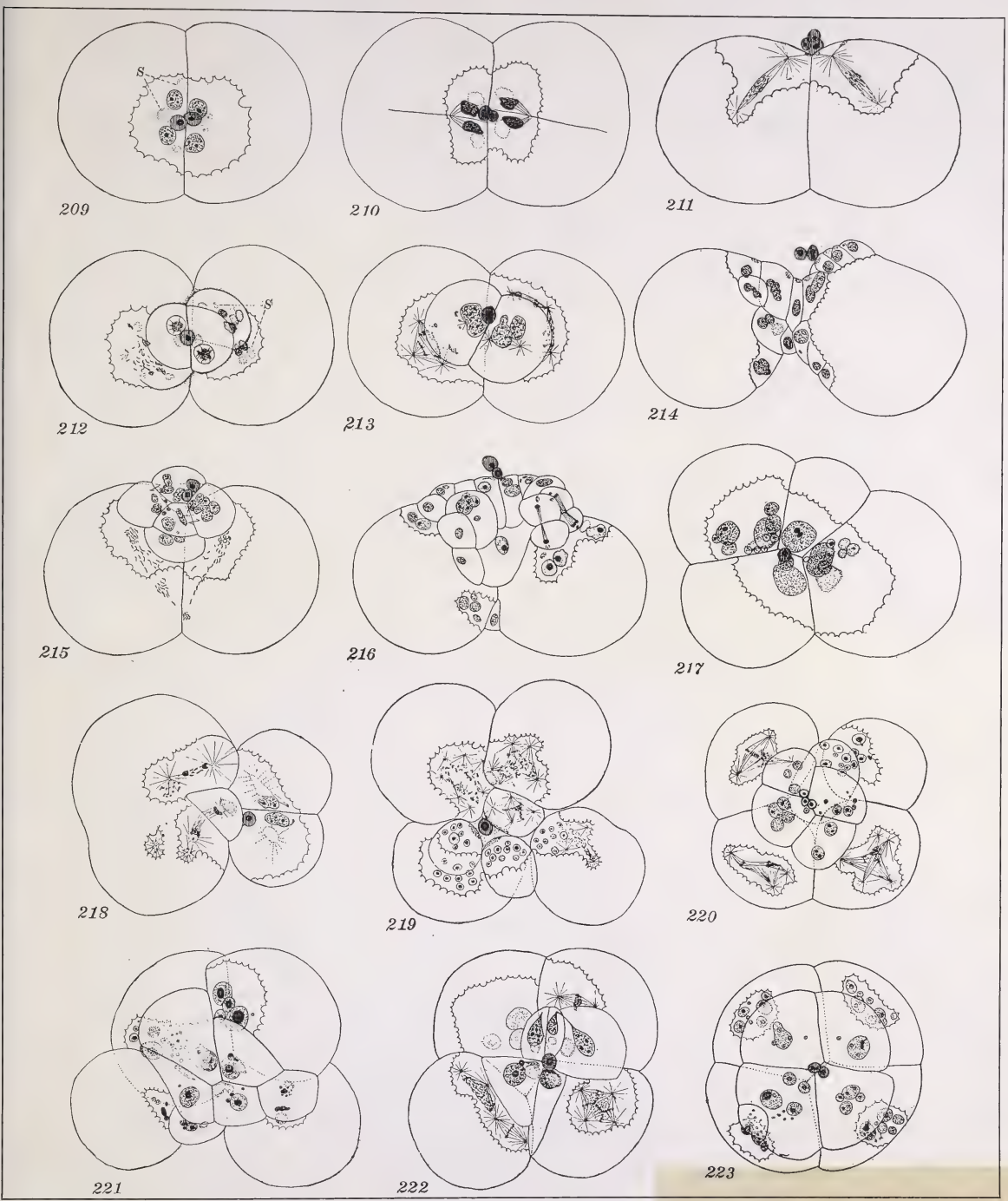
Fig. 219. Exp. 867: 8 per cent. $MgCl_2$, ¾ hr., normal 6½ hrs.; the 3d cleavage was very irregular and has given rise to a large number of karyomeres, and spheres which are in division in three quadrants of the egg.

Fig. 220. Exp. 972: 2 per cent. NaCl, 16 hrs., normal 24 hrs.; second quartet formation; karyomeres or polyasters in each of the cells.

Fig. 221. Exp. 814: 2 per cent. NaCl, 1 hr., normal 17 hrs.; second quartet formation; karyomeres and spheres in every cell.

Fig. 222. Exp. 828: 1 per cent. NaCl, 2 hrs., normal 6½ hrs.; second quartet formation; karyomeres and polyasters in every cell.

Fig. 223. Exp. 972: 2 per cent. NaCl, 16 hrs., normal 24 hrs.; karyomeres (and asters) in every cell; all nuclei are vesicular and contain achromatin as well as chromatin.



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HYPERTONIC SEA WATER


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